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Alfalfa resistance to the potato leafhopper: deciphering the resistance mechanism and updating management guidelines

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Alfalfa resistance to the potato leafhopper: Deciphering the resistance mechanism and updating management guidelines

by

Stephen Alan Lefko

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Graduate College
Iowa State University

This is to certify that the Doctoral dissertation of

Stephen Alan Lefko

has met the dissertation requirements of Iowa State University

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Major Professor

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For the Major Program

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For the Graduate College

Dedication

This work is lovingly dedicated to my absolute best friend and incredible wife Kimberly.
Your untiring support and patient concessions fueled this
progress toward the goals we have in common.

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ABSTRACT

In 1997 several seed companies released alfalfa products that were marketed as resistant to the potato leafhopper, (*Empoasca fabae* Harris), the key pest of this crop in the Midwest and northeastern United States. The objectives of this research were to investigate the mechanism of insect resistance in leafhopper-resistant alfalfa and to determine if potato leafhopper-resistant alfalfa would require updated pest-management guidelines. It was determined that an antixenotic mechanism functioned at a plant or stem level, and without a choice, leafhoppers could feed as much on resistant alfalfa compared with susceptible alfalfa. Antixenosis was largely a function of insect behavior. The antixenotic mechanism detected in lab studies was not detected at the field-plot scale. The densities of potato leafhopper adults and nymphs were similar among plots of one susceptible alfalfa and four leafhopper-resistant alfalfas during three years of sampling. Cage studies were used to compare the potential for loss from potato leafhopper and leafhopper population growth potential on resistant and susceptible alfalfas. Resistant alfalfas had a lower potential for loss, compared with susceptible alfalfa, beginning with the second cutting of the seeding year. Moreover, similar numbers of potato leafhopper nymphs were produced on susceptible and resistant alfalfas. We used a stand tolerance concept to describe why these new alfalfas have a greater yield potential compared with susceptible alfalfa when the leafhopper number is high; the leafhopper population-damage-potential might be reduced if leafhoppers aggregate on a fraction of the stand. Stand tolerance implies the interplay of more than one resistance mechanism, but emphasizes the impact this tactic will have on pest management by raising the economic injury level. We calculated economic thresholds for both alfalfa types and found that the threshold was similar for both alfalfa types when the alfalfa was young. The

threshold in susceptible alfalfa went from 8 adult leafhoppers per 10 seeps during the seeding year up to 33 leafhoppers per 10 sweeps in subsequent years. The threshold in tolerant alfalfa went from 8 per 10 sweeps during the first cutting of the seeding year up to 80 per 10 sweeps in subsequent cuttings and years.

CHAPTER 1. GENERAL INTRODUCTION

Dissertation Organization

The general introduction contains an overview of alfalfa production and its importance as a forage crop followed by the justification and objectives for this research. This is followed by a literature review section that provides citations and background information on the biology and ecology of the potato leafhopper, leafhopper population dynamics, and previous work investigating alfalfa mechanisms of resistance to the potato leafhopper. Chapters 2 through 6 are individual manuscripts submitted to scientific journals. Chapter 2 investigates the effect of alfalfa resistance on the insect community, and especially the potato leafhopper. An improved technique for quantifying potato leafhopper feeding is described in chapter 3. Chapter 4 reports findings from a laboratory study that investigated leafhopper feeding on resistant alfalfa and the presence of an antixenotic resistance mechanism. The fifth chapter is probably the most important chapter. An alfalfa stand-tolerance concept is introduced in this chapter and the effect of stand tolerance on the economics of leafhopper management is discussed in detail. Findings from a study of the differential effects of potato leafhopper feeding on tolerant and susceptible alfalfas are presented in chapter 6. The final chapter contains a general conclusion for chapters 2 through 6. References cited in Chapter 1 are listed after the general conclusion.

Introduction

One of the most recent commercial advancements in alfalfa (*Medicago* spp.) pest management targets a key pest in the Midwest and northeastern United States, the potato leafhopper, *Empoasca fabae* (Harris). This ubiquitous pest is an assimilate remover that causes stunting and leaf chlorosis in alfalfa and other crops. Economic loss from potato

leafhopper is best linked to reductions in alfalfa biomass and less so of forage quality (Hutchins et al. 1989a). In 1997 several seed companies released alfalfa varieties that were advertised as resistant to the potato leafhopper.

The potential for leafhopper-resistant alfalfa was first recognized in the 1980's when Sorensen et al. (1985, 1986) released perennial alfalfa with multiple pest resistance. Releases were referred to as glandular-haired alfalfa. Use of the term glandular-haired alfalfa may be a relic from the work of Shade et al. (1979) who showed that hairs on an annual alfalfa secreted a sticky substance that entangled small insects. At the time work began on this dissertation, researchers were unaware of the presence and relative importance of the 3 resistance mechanisms outlined by Painter (1951): antibiosis, nonpreference (often used synonymously with antixenosis), and tolerance.

Much attention has focused on the dense pubescence found on these new leafhopper-resistant alfalfas and most studies have been restricted to the laboratory. Many reports have emphasized the importance of pubescence in conferring resistance to the potato leafhopper, and they usually cite antibiosis or antixenosis resistance mechanisms (Brewer et. al. 1986a; Brewer et al. 1986b; Elden and Elgin 1992; Elden and McCaslin 1997). Tolerance has received little attention by researchers. This mechanism seems important because Hogg et al. (1998) and Lefko et al. (1997) showed the population density of potato leafhopper was similar between resistant and susceptible alfalfa, yet there was a yield advantage from resistant alfalfa when the leafhopper densities were high.

Understanding the resistance mechanism in leafhopper-resistant alfalfa and its effects on the insect community is vital to its incorporation as an economically and environmentally important tactic in forage pest management. Not knowing the true resistance mechanism will

make predicting population trends of potato leafhopper, and possibly other pests impossible, and will delay researchers from updating alfalfa pest management guidelines if necessary.

Objectives

The specific objectives of this dissertation were:

1) To determine the effect potato leafhopper-resistant alfalfa has on the insect community.
2) To investigate the presence and relative importance of antibiosis, antixenosis, and tolerance in leafhopper-resistant alfalfa.
3) To determine if the alfalfa yield response to potato leafhopper was different between resistant and susceptible alfalfa, and if so, what growth characteristics allowed leafhopper-resistant alfalfa to outperform susceptible alfalfa.

Literature Review

Biology of the potato leafhopper

The potato leafhopper feeds on plant assimilates, and is a pest of alfalfa grown in the Midwest and Eastern United States. The adults are light green, wedge-shaped, and measure about 3.0 mm to 3.5 mm in length. Eggs are laid singly in plant tissue and hatch in about 6 to 10 days. The potato leafhopper has 5 instars and the nymphal stage lasts approximately 2 weeks. Nymphs look similar to the adults except that their wing pads are not easily distinguishable until the third instar. Adult leafhoppers live for approximately 4 weeks (Metcalf and Metcalf 1993). The leafhopper lacks host specificity and over 200 plants have been identified as suitable hosts for feeding (Poos and Wheeler 1949).

Potato leafhopper population dynamics

The potato leafhopper migrates to the upper Midwest and Northeast each year from overwinter locations in the Gulf States (Pienkowski and Medler 1964). There is evidence

that airflows may allow for a return migration, but to date, there is no proof that this occurs (Taylor 1989). Leafhoppers usually arrive in Iowa during mid May or early June, near the end of the first growth interval.

Leafhoppers are usually most dense in alfalfa during the second growth interval of a 3-cut harvest schedule which is commonly used in Iowa (DeGooyer et al. 1998a). However, DeGooyer et al. (1998a) showed that the population density of potato leafhopper in Iowa alfalfa could exceed the economic threshold during all 3 growth intervals of a 3-cut system.

After the initial migration of leafhoppers into alfalfa fields, temperature (Hogg 1985; Simonet and Pienkowski 1980), alfalfa harvest (Simonet and Pienkowski 1979), and a fungal pathogen (*Zoopthora radicans* (Brefeld) Batko) (Hunter 1991) are the major factors responsible for regulating the population size.

Potato leafhopper injury to alfalfa

Injury to alfalfa by the potato leafhopper is caused by the deposition of salivary enzymes and not the mechanical damage caused by the insertion of their stylets (Ecale and Backus 1995a). Leafhopper feeding causes alfalfa phloem cells to collapse and reduces xylem tissue in quantity and size; this damage to the vascular systems might be responsible for the symptoms of potato leafhopper, namely, hopperburn and stunting (Ecale and Backus 1995b).

Hopperburn is used to describe the chlorotic leaf-tips of alfalfa, a symptom of potato leafhopper feeding. Hutchins et al. (1989a) found that chlorotic, or hopperburned, leaves provided ruminants nutrition equal to that found in non-chlorotic leaves, and that leafhopper feeding actually enhanced total herbage forage quality by increasing the leaf to stem ratio. However, hopperburned leaves tend to senesce earlier than healthy leaves and Fuess and

Tesar (1968) found that leaf-drop, due to natural leaf senescence, was increased by 1.2 Mg ha^{-1} when the harvest maturity was delayed from 10 to 100% bloom.

Although hopperburn is the most visible symptom of feeding, stunting is the most economically important symptom. Womack (1984) showed how leafhopper feeding reduced transpiration and photosynthetic rates, and Hutchins et al. (1989b) showed how feeding translated into reduced (stunted) stem length and lower biomass production per unit area. Hutchins et al. (1990a) have prepared a thorough review of the physiological and economic impacts of potato leafhopper feeding on alfalfa.

Producers can prevent the economic consequences of potato leafhopper in alfalfa by scouting (Bechinski 1994) and using an economic threshold (Pedigo et al. 1986). Scouting is a sampling program accompanied by a set of decision guidelines, usually an economic threshold. Cuperus et al. (1983) modified natural population densities of the potato leafhopper in established alfalfa and measured the yield response to this pest. Using these data, they calculated economic thresholds of 0.32, 0.40, and 0.50 leafhoppers per pendulum sweep when the alfalfa was 5, 12, and 17cm tall. During the same time period, Smith and Ellis (1993) reported that stunting and hopperburn began at 0.09 adult leafhoppers per stem and dry weight wasn't reduced until there were 0.17 leafhoppers per stem. Hutchins and Pedigo (1990b) calculated loss coefficients for the potato leafhopper in alfalfa, and later, DeGooyer et al. (1998b) used these coefficients to calculate economic thresholds for three relative sampling techniques. Using the sweepnet technique, they economic threshold as 1.5 adult potato leafhoppers per sweep.

Alfalfa resistance to potato leafhopper

Alfalfa resistance to the potato leafhopper is an innovative pest management tactic. This is evident from the relatively few number of articles published in the scientific literature.

Alfalfa resistance to the potato leafhopper was discovered when different alfalfa lines showed variable degrees of the hopperburn symptom. Jarvis and Kehr (1966) screened 75 alfalfa clones for resistance to potato leafhopper and found that equal numbers of nymphs did not cause the same degree of hopperburn. They suggested that some lines were resistant to hopperburn. Similarly, Kindler et al. (1973) screened alfalfa clones and found variable levels of alfalfa resistance to hopperburn, however, there was a poor relationship between hopperburn and final alfalfa yield. Moreover, alfalfa that is resist hopperburn provides no greater value because hopperburned leaves have the same forage value as leaves without hopperburn (Hutchins et al. 1989a). Soon though, alfalfa breeders began to register alfalfa that was described as resistant to the potato leafhopper (Thompson et al. 1974; Kehr and Manglitz 1984; and Elden and Elgin 1989). There was no thorough explanation of the mechanism of resistance, however, the role of dense simple pubescence was emphasized in these reports. Researchers, as early as Grasnovsky (1928), described how pubescence on alfalfa could confer resistance to the potato leafhopper. Later, Elden et al. (1986) showed that pubescence was a heritable trait and described how it could be selected for in alfalfa breeding programs.

During the same time period, alfalfa researchers were investigating the potential for insect resistance using a glandular hair trait in annual (Shade et al. 1975, 1979) and perennial

alfalfa (Kreitner and Sorensen 1979). Shade et al. (1975) found that erect glandular hairs on several annual Medicago spp. entrapped and killed alfalfa weevil larvae. Later, Shade et al. (1979) found these hairs had the same effect on the nymphal stage of potato leafhoppers. There was no way, however, to utilize this favorable trait found in annual alfalfa for the development of agronomically acceptable perennial alfalfa. Kreitner and Sorensen (1979) reported finding erect glandular hairs on the perennial diploid *M. prostrata* Jacquain and a perennial tetraploid *M. sativa* subsp. *praefalcata* (Sinskaya) Gunn. Kitch et al. (1985) described that the glandular hair trait was plygenic and complex, and although the trait was stable, *M. prostrata* was not a favorable forage species. Kreitner and Sorensen (1979) produced F_1 progeny from a cross using *M. prostrata* and an *M. sativa* hay type alfalfa. This successful cross marked great progress towards the development of perennial agronomic alfalfa with the glandular hair resistance trait. Elden and McCaslin (1997) provide a more thorough account of these events.

The role these different annual and perennial, and diploid and tetraploid alfalfas played in the development of commercial alfalfas resistant to the potato leafhopper is unclear in the literature. However, most of the commercial alfalfa that is resistant to the potato leafhopper is probably a result of breeding programs using perennial glandular-haired alfalfa, similar to the one described by Kreitner and Sorensen (1979). Although probably impossible to document, the sources of resistance in commercial lines are probably not the same as those described as resistance to yellowing in reports before 1970. Therefore, it is wrong to assume that the mechanism(s) that makes alfalfa resistant to the potato leafhopper has remained the same throughout the last 80 years.

Alfalfa with resistance to the potato leafhopper was released commercially in 1997 and most research and commercial organizations called it glandular-haired alfalfa. This name was probably used because of the reports by Shade et al. (1975, 1979) on annual glandular-haired alfalfa and maintained its popularity because there was no better explanation for the resistance mechanism. Still, there is no evidence that commercial (perennial glandular-haired tetraploid) alfalfa has glandular hairs that entrap small insects (Brewer et. al. 1986a; Elden and McCaslin 1997).

To date, most of the literature reporting on the mechanism(s) or resistance in commercial (perennial tetraploid) leafhopper-resistant alfalfa are limited to laboratory studies, and most of these report data generated from alfalfa breeding techniques. Therefore, there are no comprehensive reports that qualify or prioritize the presence of antibiosis, nonpreference (antixenosis), or tolerance in these alfalfas.

Brewer et. al. (1986a) compared levels of antibiosis and nonpreference of 3 species of glandular-haired alfalfa to 1 nonglandular hay type of alfalfa. In general, clones of glandular-haired species showed ovipositional nonpreference. However, the level of oviposition in some clones with dense glandular hairs did not differ from oviposition in the nonglandular hay-type. The authors also took measures of adult survival and leaf wilting in no choice tests. In general, leaf wilting was greater on the nonglandular-haired compared to glandular-haired alfalfa, and adult mortality was greater on glandular-haired compared to the nonglandular-haired alfalfa. The authors did not detect an entrapment mechanism similar to the mechanism Shade et al. (1979) found in annual glandular–haired types. In a separate study, Brewer et al. (1986b) examined the anatomical features of glandular-haired clones with different levels of resistance to the leafhopper. They cited glandular hairs, stems with

small cross-sectional areas, and highly lignified tissue as sources of resistance. Elden and McCaslin (1997) showed significant but not strong correlations between the density of glandular hairs and resistance to potato leafhopper in 19 clones of glandular-haired alfalfa. Estimates of nymphal mortality ranged from 0 to 33%, and they reported 13 to 96% mortality of adult leafhoppers in no-choice cage tests. They also stated that glandular hairs on these perennial clones did not entrap leafhoppers, and suggested that an unexplained resistance mechanism may exist.

Although antibiosis and nonpreference have been the focus of most studies, tolerance has received little attention. It seems this mechanism deserves more attention because Hogg et al. (1998) and Lefko et al. (1997) showed the population density of leafhopper was similar between resistant and susceptible alfalfa, yet there was a yield advantage from resistant alfalfa when the leafhopper densities were high.

CHAPTER 2. INSECT COMMUNITIES IN SUSCEPTIBLE AND RESISTANT ALFALFA WITH AN EMPHASIS ON POTATO LEAFHOPPER

A paper submitted to the Journal of Economic Entomology

Stephen A. Lefko, Larry P. Pedigo, and Marlin E. Rice

Abstract

In 1997 several companies released alfalfa (*Medicago* spp.) varieties that were marketed as resistant to potato leafhopper, *Empoasca fabae* (Harris). To date, there are no studies comparing the insect communities in potato leafhopper-resistant alfalfa to those found in susceptible alfalfa. This information is critical in order to determine how pest and potential natural enemy populations will respond to the mechanism of resistance in new resistant varieties. Potential insect-alfalfa interactions such as multiple pest resistance, pest or natural enemy displacement, or no interactions at all, may change pest management guidelines in potato leafhopper-resistant alfalfa. Eighteen insect species, including pests and potential natural enemies, were sampled in one susceptible (645®) and four leafhopper-resistant glandular-haired alfalfas during 1996 and 1997. Only 4 pest species were significantly more dense on a given date in the susceptible alfalfa compared with any resistant variety throughout the 2-yr study. Six pest species were significantly more dense in resistant varieties compared with the susceptible control in 17 species-by-date-by-variety comparisons, and the same species were never significantly less dense in susceptible alfalfa. Adult potato leafhopper density was significantly greater in the susceptible control compared with all resistant varieties in only 11 of the 140 date-by-variety comparisons. The density of potato leafhopper nymphs was significantly greater in the susceptible control in only 4 of the 92 date-by-variety comparisons. Potential insect natural enemies were significantly more

dense in resistant alfalfa compared with the susceptible control in 11 species-by-date-by-variety comparisons, and significantly less dense in only one. Results from this study show that the insect communities in these first-generation leafhopper-resistant alfalfa varieties likely will not differ from those found in susceptible alfalfa. Potato leafhopper, and several other pests and potential natural enemies, show no preference for occupying field plots of susceptible alfalfa, compared with potato leafhopper-resistant varieties of alfalfa. This information was used to develop a hypothesis on how the resistance mechanism(s) may function in these varieties.

Introduction

Alfalfa (*Medicago* spp.) serves as host to an abundance of phytophagous insect species. One of these, the potato leafhopper, *Empoasca fabae* (Harris), is considered the primary pest of alfalfa in the north central and northeastern United States. This ubiquitous pest is an assimilate remover and causes stunting and leaf chlorosis in alfalfa. Economic loss from potato leafhopper is best linked to reductions in alfalfa biomass and less so of forage quality (Hutchins et al. 1989).

In 1997 several seed companies sold alfalfa varieties that were advertised as resistant to the potato leafhopper. There are few reports of yield benefits from leafhopper-resistant alfalfa. Hogg et al. (1998) and Lefko et al. (1997) showed slightly lower yields compared to susceptible alfalfa when leafhopper pressure is low, and a yield advantage when pest pressure is high and populations were left unmanaged. These two studies included varieties used in the current study. These alfalfa varieties have more dense pubescence than susceptible alfalfa and at least one hair type that is visibly different from the simple hairs (Elden et al. 1986) found on most hay-type alfalfa. This hair type has been described in the potato

leafhopper–resistant alfalfa literature as a glandular hair (Brewer et al. 1986; Elden and McCaslin 1997). An assumption is that these new commercial varieties are related to lines evaluated in these studies and likely share some level of a common resistance mechanism.

Recent studies of perennial glandular-haired alfalfa emphasize the importance of glandular hairs in conferring resistance to potato leafhopper. Brewer et. al. (1986) compared levels of antibiosis and nonpreference of three species of glandular-haired alfalfa to one nonglandular hay type of alfalfa. In general, clones of glandular-haired species showed ovipositional nonpreference. However, the level of oviposition in some clones with dense glandular hairs did not differ from oviposition in the nonglandular hay-type. The authors took measures of adult survival and leaf wilting in no choice tests. In general, leaf wilting was greater on the nonglandular-haired compared to glandular-haired alfalfa, and adult mortality was greater on glandular-haired compared to the nonglandular-haired alfalfa. The authors did not detect an entrapment mechanism similar to the mechanism Shade et al. (1979) found in annual glandular–haired types. Elden and McCaslin (1997) showed significant but not strong correlations between the density of glandular hairs and resistance to potato leafhopper in 19 clones of glandular-haired alfalfa. Estimates of nymphal mortality ranged from 0 to 33%, and they reported 13 to 96% mortality of adult leafhoppers in no-choice cage tests. They also stated that glandular hairs on these perennial clones did not entrap leafhoppers, and suggested that an unexplained resistance mechanism may exist. From their study, it appears that the "stickiness" of glandular hairs in these commercial varieties may have been lost in the breeding process (Elden and Elgin 1987), or maybe never existed in the perennial parent lines of these varieties. It may be that, for lack of a better

explanation, the resistance mechanism in commercial leafhopper-resistant alfalfa varieties is believed to be conferred by sticky, glandular hairs.

It is likely that commercial varieties are closely related to the clones used in these and other recent studies, but to date, the resistance mechanism of commercial varieties is largely unknown. Understanding the resistance mechanism of glandular-haired alfalfa and its effects on the insect community is vital to its incorporation as an economically and environmentally important tactic in forage pest management. Not knowing the true resistance mechanism will make predicting population trends of potato leafhopper and possibly other pests difficult. This fact has far-reaching pest–management implications.

To date, there are no reports of how insect communities in field plantings of potato leafhopper–resistant alfalfa will compare to those in susceptible alfalfa. There are several potentially important changes to the insect communities that could result from using these resistant varieties. Potato leafhopper–resistant alfalfa may impart resistance to other occasional pests of alfalfa. These pests may die or be displaced to other nearby susceptible alfalfa fields. Death or displacement of these species could result in fewer insect natural enemies, thus causing outbreaks of secondary insect pests.

The objective of this study was to determine the effect potato leafhopper–resistant alfalfa has on the insect community by sampling insects in field plantings of resistant and susceptible alfalfa.

Materials and Methods

Two study areas near Ames and Chariton, IA were planted with 4 potato leafhopper-resistant, glandular-haired, alfalfa varieties and 1 susceptible variety. These locations were chosen based on environmental differences and histories of different pest populations.

Resistant alfalfa varieties included ABI AmeriGuard 301®, CENEX Trailblazer®, Pioneer Hi-Bred 5347LH®, and XAE49, a Pioneer Hi-Bred experimental line. The susceptible control variety was Garst 645®. The 5 varieties were arranged as a randomized complete block, with 4 replications. Both locations were planted during the fourth week of April 1996 and treated with eptam 1 week before planting to reduce weed competition. Each plot measured 7.6 m long by 6.1 m wide and was planted using a single-row planter; these dimensions were determined by the quantity of seed available. Rows were spaced 20 cm apart, and the planter was calibrated to 0.19 g seed per meter (10 lb per acre). The plots at both sites were located within 0.6-ha fields of Defiant®, a susceptible variety of alfalfa.

Alfalfa was harvested twice during 1996 and 3 times during 1997. Cutting occurred when the stand reached approximately the 0.1-bloom stage of development. Both fields were cut during the second week of July and the last week of August in 1996, and during the first week in May, first week in July, and second week in August in 1997.

Sampling methods were similar in 1996 and 1997. Insect communities were sampled using a 38 cm-dia. sweepnet. A sampling unit consisted of 12 pendulum sweeps of the alfalfa canopy in a plot. Sweep samples were taken lengthwise through all plots, and the area sampled within each plot alternated by sampling date. Samples were bagged and kept frozen until processing. There were 17 sampling dates at the Ames location, 5 in 1996 and 12 in 1997. There were 18 sampling dates at the Chariton location, 8 in 1996 and 10 in 1997. Plots were sampled on a regular basis from July through August in 1996 and from May until September during 1997. The 18 insect species sampled in this study are listed in Table 1. These species constitute the major pests and potential insect natural enemies commonly

found in alfalfa in the region but do not represent the entire alfalfa-insect community (Pimentel and Wheeler 1973).

The density of each insect species was compared among all varieties on each sampling date. These data are presented as the number significant comparisons of each resistant alfalfa compared to the susceptible alfalfa over the two-year period. Potato leafhopper and pea aphid, *Acyrthosiphon pisum,* (Harris) were also analyzed by alfalfa regrowth interval, which included multiple sampling dates and a repeated measures analysis. Analysis of variance was used to test for significant differences of insect species densities among alfalfa varieties. If the analysis of variance was significant ($P > 0.05$), means were separated using least significant difference analysis ($P > 0.05$) (SAS 1990). This procedure was used on species-by-date and species-by-regrowth interval data from both locations.

Results

All insects included in the study were detected at both locations over the 2-year study. Pest species were more frequent than beneficial species at both locations as shown in Table 1. In most instances, the density of insect pest and potential natural enemy species rarely differed among resistant and susceptible alfalfa varieties.

Date-by-Variety Comparisons of Insect Density

Only 4 pest species; potato leafhopper, differential grasshopper, pea aphid, and meadow spittlebug, were significantly more dense on a given date in susceptible alfalfa than in any resistant variety throughout the 2-yr study (Table 1). These differences, however, were rare among sampling dates, and a trend for greater densities of these insects in susceptible alfalfa was not apparent.

Some insects were only significantly greater in a resistant alfalfa variety compared with the susceptible variety. Green cloverworm, tarnished plant bug, alfalfa plant bug, plant bug nymphs, and green stink bug, were significantly more dense in resistant varieties than in the susceptible control in 17 date-by-variety comparisons, and never less dense in susceptible alfalfa. Additionally, the densities of pea aphid and differential grasshopper were more often significantly greater in any resistant variety compared with the susceptible one. Although differences existed, there was no trend for increased differential grasshopper density in resistant alfalfa. Pea aphid, however was significantly greater in any resistant variety compared with the susceptible control in 21.5% of the date-by-variety-comparisons.

There was no obvious trend for increased density of potato leafhopper in susceptible alfalfa compared with the resistant alfalfas. Adult potato leafhopper density was significantly greater in the susceptible alfalfa in only 7.9% of the date-by-variety comparisons. Likewise, potato leafhopper nymph density was significantly greater in susceptible alfalfa in only 4.3% of the date-by-variety comparisons. Adult and nymph potato leafhoppers were never more dense in any resistant variety compared with the susceptible control (Table 1).

The density of potential insect natural enemies was significantly greater in susceptible alfalfa in 11 species-by-date-by-variety comparisons and significantly less in only 1 (Table 1). The only insects for which significant differences were recorded were the common damsel bug and *Coleomegilla maculata* (DeGeer). The densities of common damsel bug and *C. maculata* were significantly greater in the susceptible alfalfa in 3.1% and 5.4% of the date-by-variety comparisons, respectively.

Comparisons of Insect Density by Alfalfa Growth Interval

Potato leafhopper and pea aphid are 2 species that are important economically and that had more frequent differences in density in the date-by-variety comparisons. These species also showed some differences among alfalfa type when analyzed by alfalfa growth interval. The density of adult potato leafhopper was numerically higher in the control plot compared with all resistant plots during three growth intervals at the Ames location (Table 2). Conversly, the density of potato leafhopper was higher in all resistant plots compared with the control plot during the first cutting of 1996 at the Chariton location. The only comparison with significance was between the control and XAE49 during the second cutting of 1997, and leafhoppers were more dense in the control. There was one comparison of nymph densities that yielded significant results. The nymph density was significantly greater in the AmeriGuard 301 compared with the susceptible control during the third growth interval of 1997 at Chariton. Other than these two instances of significance, potato leafhopper densities between resistant and susceptible alfalfa did not differ during the alfalfa regrowth periods at either location.

The density of pea aphid was very often more dense in resistant alfalfa compared with the susceptible control (Table 2). Pea aphid was significantly more dense in Trailblazer, 5347LH, and XAE49 compared with the susceptible control during the second alfalfa growth interval of 1996 at Chariton (Table 2). Similarly, the aphid density was significantly higher in Trailblazer and XAE49 compared with the susceptible control during the second growth interval of 1997 at the Chariton location.

Discussion

The insect communities in moderate-sized field plots of resistant and susceptible alfalfa seldom differed during the first 2 years of alfalfa growth. But, there was a trend towards increased densities of pea aphid in potato leafhopper–resistant alfalfa compared with the susceptible alfalfa. There is no explanation for this phenomenon. Therefore, producers should not expect reduced insect densities in the resistant varieties used in this study.

Deductions can be made about the resistance mechanism from data reported in this study. Results show that the nonpreference mechanism that functioned in choice studies in earlier research did not function among field plots of resistant and susceptible alfalfa. The plots used in this study could be considered large free-choice tests. Assuming immigration of leafhoppers into resistant plots did not equal emigration to an alternative location, nonpreference did not seem to function among varieties (plots). This contradiction may be explained by differences in methodology. Roof et al. (1976), Brewer et al. (1986), Elden and Elgin (1992), and Elden and McCaslin (1997) showed leafhopper nonpreference in alfalfa using different field and laboratory choice and no-choice studies. These studies show nonpreference; however, they may be confounded by the proximity of available leafhopper food and oviposition resources. Laboratory choice studies caged leafhoppers on clones in close proximity; similarly, field studies used natural infestations on clones in space-planted designs. A limited number of host choices and an incomplete set of environmental stimuli may have confounded insect behavior in these studies. Those studies may not have adequately provided potato leafhoppers with the all of the choices available in a field setting, especially field selection after migration (Pienkowski and Medler 1964). If nonpreference

functions in commercial leafhopper-resistant alfalfa varieties, it may be important only on a plant basis and not among fields of resistant and susceptible alfalfa.

Elden and McCaslin (1997) caged leafhoppers on individual plants and detected high levels of antibiosis in some clones. Antibiosis was correlated with the density of glandular hairs. However, they did not detect insects entangled in glandular hairs. A similar level of antibiosis may exist in certain alfalfa genotypes in these commercial varieties. Antibiosis may not be detected however unless alfalfa clones are used in a no-choice study, and it may not function until the entire (field) population of alfalfa expresses a similarly high level. The present study does not rule out an antibiotic resistance mechanism. But, unless immigration into resistant alfalfa plots equaled emigration or mortality in the same plots throughout the present study, these data suggest that antibiosis may be less important than previously believed, especially in a production situation.

An alfalfa variety that supports a leafhopper population equal to that found in a susceptible variety, and results in higher yield than the susceptible variety, can be considered tolerant (Painter 1951). Unless immigration equaled emigration or mortality, data in the present study show ½ of the tolerance equation: populations of resistant alfalfa supporting a leafhopper population equal to that found in a susceptible variety. If future research shows yield advantages from using these resistant varieties, which is the other ½ of the equation, then tolerance of an individual plant or a plant population may be one contributing mechanism of resistance.

Tolerance as a predominant mechanism of resistance has many management implications. Alfalfa that tolerates the potato leafhopper may not meet producer expectations, especially if new varieties are marketed as resistant without an explanation that

the insect community may remain the same. Moreover, current economic thresholds range near 0.5 adult leafhoppers per sweep when alfalfa is < 17 cm tall (Cuperus et al. 1983). Although new resistant alfalfa probably decrease stand injury from potato leafhopper, there likely exists a high population density that will result in an economic loss if not managed. Until new thresholds are calculated, producers using thresholds developed on susceptible alfalfa may make unnecessary insecticide applications and consequently never realize the full potential of potato leafhopper–resistant alfalfa.

Future work aimed at detecting the mechanism of resistance in these commercial varieties should examine all resistance mechanisms. Limiting experiments to testing for antibiosis, nonpreference, or tolerance, may produce results that do not accurately describe how populations of potato leafhopper will react under field conditions. Consequently, alfalfa producers may misinterpret the potential added value of these new varieties.

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Table 1. Date-by-variety comparisons of insect density

Insects sampled	Ames, Iowa				Chanton, Iowa			
	No. dates detected	No. D by V[a]	Sus. greater[b]	Res. greater[c]	No. dates detected	No. D by V[a]	Sus. greater[b]	Res. greater[c]
				Pest Insects				
Potato leafhopper (adult)	16	64	5	-	19	76	6	-
Potato leafhopper (nymph) *Empoasca fabae* Harris	11	44	-	-	12	48	4	-
Alfalfa weevil (adult)	7	28	-	-	7	28	-	-
Alfalfa weevil (nymph) *Hypera postica* Gyllenhal	9	36	-	-	11	44	-	-
Alfalfa caterpillar *Colias eurytheme* Biosduval	9	36	-	-	16	64	-	-
Green cloverworm *Hypena scabra* Fabricious	15	60	-	7	11	44	-	-
Tarnished plant bug *Lygus lineolaris* Palisot de Beauvios	16	64	-	-	19	76	-	2
Alfalfa plant bug *Adelphocoris lineolatus* Goeze	13	52	-	-	16	64	-	3
Plant bug nymph (spp.)	15	60	-	1	18	72	-	3
Green stink bug *Acrosternum hilare* Say	13	52	-	1	16	64	-	-
Red legged grasshopper *Melanoplus femurrubrum* De Geer	15	60	-	-	15	60	-	-
Differential grasshopper *Melanoplus differentialis* Thomas	13	52	-	1	16	64	1	3
Pea aphid *Acyrthosiphon pisum* Harris	17	68	1	4	19	76	-	27
Meadow spittlebug *Philaenus spumarius* Linnaues	12	48	-	-	16	64	1	-
				Potential insect natural enemies				
Common damsel bug *Reduviolus americoferus* Carayon	14	56	1	-	18	72	3	-
Minute pirate bug *Orius tristicolor* White	12	48	-	-	13	52	-	-
Coleomegilla maculata	15	60	7	1	17	68	-	-
Sevenspotted lady beetle *Coccinella septempunctata* Linnaeus	6	24	-	-	12	48	-	-
Thirteen spotted lady beetle *Hippodamia tredecimpunctata tibialis* Say	5	20	-	-	7	28	-	-
Convergent lady beetle *Hippodamia convergens* Guerin-Meneville	9	36	-	-	5	20	-	-
Green lacewing *Chrysoperla carnea* Stephens	4	16	-	-	9	36	-	-

[a] The number of date (D)-by-variety (V) comparisons of the insect mean among all varieties.

[b] The number of camparisons when the mean for the insect was significantly greater in the susceptible compared with any resistant variety ($P > 0.05$, ANOVA).

[c] The number of camparisons when the mean for the insect was significantly greater in any resistant variety compared with the susceptible ($P > 0.05$, ANOVA).

Table 2. Mean insect densities and standard errors for alfalfas by regrowth interval.

Growth interval	No. sampling dates	Insects	Insects per 12 sweeps[a]				
			AmeriGuard 301, ABI	Trailblazer, CENEX	5347LH, Pioneer	XAE49, Pioneer	645, Garst
			Ames 1996				
1st	2	PLH adult	36.1 ± 9.3	26.8 ± 9.2	57.6 ± 10.9	40.4 ± 7.7	59.9 ± 18.5
		PLH nymph	20.1 ± 8.6	11.8 ± 6.6	18.4 ± 4.3	15.8 ± 6.2	20.9 ± 7.8
		Pea aphid	1.5 ± 0.4	2.5 ± 0.7	1.5 ± 0.5	2.9 ± 1.0	3.5 ± 0.9
2nd	3	PLH adult	6.1 ± 1.3	5.7 ± 1.0	7.6 ± 0.9	8.7 ± 1.5	10.7 ± 2.6
		PLH nymph	0.6 ± 0.3	0.7 ± 0.3	0.8 ± 0.3	0.3 ± 0.2	1.3 ± 0.5
		Pea aphid	1.1 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	1.1 ± 0.4	0.8 ± 0.3
			Ames 1997				
1st	2	PLH adult	0.9 ± 0.5	1.3 ± 0.7	0.1 ± 0.1	0.6 ± 0.3	1.3 ± 0.8
		PLH nymph	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Pea aphid	6.0 ± 2.8	4.0 ± 1.0	2.8 ± 1.1	4.3 ± 1.9	4.0 ± 1.5
2nd	5	PLH adult	42.5 ± 9.2	32.1 ± 7.1	35.6 ± 7.7	32.7 ± 6.4	38.4 ± 9.4
		PLH nymph	2.1 ± 1.0	1.6 ± 0.8	1.6 ± 0.7	1.8 ± 0.7	2.6 ± 1.6
		Pea aphid	22.5 ± 8.0	31.2 ± 10.4	30.9 ± 11.1	24.6 ± 7.5	15.1 ± 6.2
3rd	5	PLH adult	21.1 ± 3.3	18.8 ± 4.1	20.6 ± 3.5	17.5 ± 3.2	24.0 ± 3.6
		PLH nymph	3.1 ± 0.9	1.6 ± 0.3	2.4 ± 0.6	1.4 ± 0.4	5.1 ± 1.7
		Pea aphid	9.2 ± 2.3	18.8 ± 4.1	14.5 ± 3.5	7.9 ± 2.2	6.2 ± 2.1
			Chariton 1996				
1st	2	PLH adult	48.3 ± 20.0	46.8 ± 17.2	49.0 ± 16.9	36.9 ± 16.8	37.0 ± 13.9
		PLH nymph	12.9 ± 5.8	11.0 ± 4.1	13.0 ± 6.1	3.6 ± 1.4	8.9 ± 3.8
		Pea aphid	4.1 ± 1.8	9.9 ± 4.4	5.8 ± 1.6	2.9 ± 1.0	2.3 ± 1.4
2nd	6	PLH adult	8.3 ± 1.0	6.3 ± 0.8	13.4 ± 2.2	7.2 ± 1.2	8.3 ± 1.5
		PLH nymph	2.4 ± 0.4	3.1 ± 0.6	1.8 ± 0.4	1.7 ± 0.4	2.8 ± 0.6
		Pea aphid	96.2 ± 20.9 bc	217.5 ± 46.1 a	194.0 ± 38.5 ab	190.0 ± 58.2 ab	23.1 ± 6.7 c
			Chariton 1997				
1st	3	PLH adult	1.2 ± 0.5	1.7 ± 0.8	0.7 ± 0.3	2.0 ± 0.7	1.2 ± 0.4
		PLH nymph	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		Pea aphid	3.9 ± 1.0	6.9 ± 2.5	8.0 ± 2.4	5.8 ± 1.5	3.6 ± 0.4
2nd	4	PLH adult	12.4 ± 3.0 ab	10.4 ± 1.7 ab	11.7 ± 2.4 ab	9.6 ± 2.1 b	18.1 ± 3.3 a
		PLH nymph	0.9 ± 0.5	0.7 ± 0.4	1.4 ± 0.7	1.4 ± 0.8	2.5 ± 1.5
		Pea aphid	14.1 ± 3.5 bc	25.3 ± 3.7 a	18.0 ± 2.6 abc	23.3 ± 4.0 ab	11.6 ± 3.0 c
3rd	3	PLH adult	6.7 ± 1.0	4.3 ± 0.9	7.9 ± 1.6	5.3 ± 0.8	8.8 ± 2.2
		PLH nymph	0.6 ± 0.3 a	0.1 ± 0.1 b	0.1 ± 0.1 b	0.1 ± 0.1 b	0.1 ± 0.1 b
		Pea aphid	2.9 ± 1.0	4.9 ± 1.6	4.8 ± 1.5	4.8 ± 1.4	2.4 ± 1.1

[a] Means followed by the same letter, or no letter, are not significantly different ($P > 0.05$, ANOVA)

CHAPTER 3. QUANTIFYING POTATO LEAFHOPPER FEEDING ON ALFALFA USING IMAGE ANALYSIS

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Abstract

The quantity of potato leafhopper (*Empoasca fabae* Harris) feeding was compared among a susceptible and resistant alfalfa using a digital image analysis technique. Treatments consisted of six different alfalfa's, Garst 645® and five clones representing a range of resistance levels from a field population of Pioneer Hi-Bred 5347LH® alfalfa. The 5347LH variety has been referred to as a glandular-haired type, and is marketed as resistant to the potato leafhopper. Each experimental unit consisted of ten adult leafhoppers caged on a single stem cutting for 24h. Honeydew that resulted from feeding was collected on filter paper at the base of each cage. The six treatments were replicated eight times in each experiment and the experiment was repeated three times. Filter papers were treated with 0.2% ninhydrin in ethanol and the quantity of stained honeydew was measured using high-resolution photography and image analysis. Image analysis procedures were used to integrate measures of honeydew-stained area and color intensity and gave precise estimates of the quantity of stained honeydew. There were no differences in the quantity of stained honeydew among the six treatments. Leafhoppers fed as much on the most resistant 5347LH clone as they did the susceptible control. This report describes the image analysis technique used to quantify leafhopper feeding.

Introduction

The potato leafhopper (*Empoasca fabae* Harris) is a key pest of alfalfa (*Medicago sativa* L.) in the upper Midwest and northeastern United States. Plant stunting and leaf yellowing, or hopperburn, are two symptoms of potato leafhopper feeding on alfalfa. These symptoms typically occur together, and while hopperburn is the most visible symptom, economic loss is most highly correlated with plant stunting (Hutchins et al. 1989).

Hopperburn has been used by entomologists to rate the severity of a potato leafhopper infestation in alfalfa since the earliest research of Granovsky (1928), Jewett (1929), and Poos and Johnson (1936). More recently, alfalfa breeders and entomologists have used hopperburn, along with other variables, to rate the degree of alfalfa resistance to potato leafhopper and help determine the mechanisms of resistance (Roof et al. 1976, Elden and Elgin 1987, Elden and Elgin 1992, Elden and McCaslin 1997). In these studies hopperburn implied leafhopper feeding, however the relationship between feeding and hopperburn was variable among alfalfa lines. Jarvis and Kehr (1966) screened 75 alfalfa clones for resistance to potato leafhopper and found that equal numbers of nymphs did not cause the same degree of hopperburn. They suggested that some lines were resistant to hopperburn. Similarly, Kindler and Kehr (1973) screened alfalfa clones and found variable levels of alfalfa resistance to hopperburn and poor relationship between hopperburn and final alfalfa yield. Brewer et al. (1986) performed comparative studies on the antibiotic and antixenotic mechanisms of perennial glandular-haired and simple-haired alfalfa on potato leafhopper. The simple-haired alfalfa was more resistant to yellowing and did not exhibit antibiosis or antixenosis. Conversely, some glandular-haired clones had high levels of antibiosis, antixenosis, or both, and showed variable levels of hopperburn, when exposed to high

feeding pressure. One hypothesis they used to describe antibiosis was reduced feeding because of a tough vascular cylinder. Another possible explanation could be that glandular hairs impeded the insect's ability to feed as usual.

It is clear from these studies that different alfalfa lines are not equally susceptible to the onset and severity of hopperburn as a result of feeding by the potato leafhopper. Moreover, these studies show that using the hopperburn symptom to estimate feeding could be misleading and may confound our understanding of the resistance mechanisms in glandular-haired alfalfa. The goal of this study was to develop an improved technique for quantifying leafhopper feeding on stem-cuttings of alfalfa.

Materials and Methods

Five individual alfalfa plants were selected from a field population of glandular-haired alfalfa, Pioneer Hi-Bred 5347LH®, that was repeatedly stressed by naturally occurring populations of potato leafhopper. These plants were selected based on a 1–9 hopperburn rating scale. This scale used similar injury criteria as the 1–5 Standard Test to Characterize Alfalfa Cultivars (McCaslin and Miller 1996) for potato leafhopper resistance except it was inverted (1, severe injury; 9, no apparent injury) and provided more intermediate resistance ratings. The five glandular-haired clones used in the study represent 1, 3, 5, 7, and 9, using this resistance rating scale. Another single plant from a leafhopper-susceptible population of Garst 645® was selected at random for the control. Each plant was cloned from stem-cuttings for use as replicates in the study. These six clones represent the treatments used in this study. All clones were maintained in the greenhouse under a 16:8 photoperiod and 25:18°C temperature regime. Also, ten clones of each plant were transplanted to the field during the summer of 1997. All plants were cut when they reached the late-bloom stage.

Cages were fashioned from inverted one-pint ice cream containers modeled after cages used by Kahn and Saxena (1984). Each cage had a 0.5-cm hole cut through the lid so the alfalfa stem could pass through and reach a water source below. Water was held in another small plastic cup and lid that was glued to the top of the ice-cream container lid. The removable cage top was the base of the ice-cream container and had the bottom cut out and replaced with fabric mesh.

The feeding experiment was run three times, once on the first regrowth of greenhouse-grown clones, once on the fourth regrowth of greenhouse-grown clones, and once on the spring 1998 regrowth of field-grown clones. Field-grown clones grew to harvest maturity twice during 1997 before the onset of winter. Cages were arranged in the growth chamber according to a randomized complete block design, with eight replications of each treatment. The photoperiod and temperature regimes were the same as the alfalfa greenhouse conditions.

One 9-cm diameter Whatman® #5 filter-paper disk was placed snugly on the bottom of each cage, and a slit was cut for the alfalfa stem to pass through. Eight stem cuttings from each treatment were caged individually and held in the chamber for 24h. This holding period allowed stems that may have been damaged during cutting to wilt and be replaced if necessary. Cuttings were taken from below the last node with a fully expanded leaf. Each cutting had three nodes and was positioned with one node below the filter paper and two above. Cages were infested after 24h with 10 adult leafhoppers per cage from greenhouse colonies. Greenhouse colonies of potato leafhopper were reared on broad bean (*Vicia faba* L.) using a photoperiod of 16:8 (L:D) and a temperature regime of 25:18°C. Colonies were re-infested with field-collected adults each summer. Leafhoppers were allowed to feed on

stem cuttings for 24h. Preliminary experiments showed that 10 leafhoppers produced adequate honeydew in 24h to complete the analysis and would die during this interval without a food source.

After the 24h feeding interval, the filter paper was removed from each cage and an atomizer was used to mist filter papers to near saturation with 0.2% ninhydrin in ethanol (Sigma, St. Louis, MO). Treated filter papers were heated immediately in a 100°C oven for 10 minutes, and honeydew droplets turned shades of violet. This staining technique was modeled after work reported by Khan and Saxena (1984) on leafhoppers feeding on rice.

A difficult part of using honeydew to estimate feeding is quantifying the stained honeydew. Paguia et al. (1980) evaluated several techniques for measuring the quantity of planthopper honeydew. These techniques included measuring the stained area on a filter paper, cutting out stained areas and weighing the samples, estimating the volume of honeydew, and estimating the color intensity of stained honeydew. The first two techniques ignored the possibility of honeydew droplets overlapping. This overlapping of droplets is shown in Figure 1. Using either of these techniques the quantity of honeydew would be underestimated. Paguia et al. (1980) concluded that estimating the color intensity gave the best results but suggested that their technique was tedious. The improved component of this study is the use of image analysis to quantify stained honeydew droplets. This technique integrates measures of the stained area and the color intensities of stained areas. It is less labor intensive than the best method outlined by Paguia et al. (1980) and is a more objective quantification than most of the techniques they described.

An image analysis technique was used to quantify the amount of honeydew that reacted with ninhydrin on each filter paper. This technique provided an indirect measure of

leafhopper feeding (Auclair 1958). Images of filter papers were captured after each of the three trials. Filter papers were position on top of a Northern Lights (St. Catharines, Ontario) light box. Images were captured by using a Silicon Graphics Incorporated Indigo model 2XZ computer that was equipped with a Galileo (Mountain View, CA) frame-grabber and a Sony DXC-3000A (Itasca, IL) color video camera. This apparatus was set to capture images at a resolution of 2500 pixels per mm^2 and was very effective at differentiating stained spots and subtle differences in color intensity.

The photographs of stained filter papers were analyzed using Noesis Visilog (St. Laurent, Quebec) image analysis software. Color images were first converted to greyscale. Next, the threshold operation was used to isolate the image of the filter paper from the light-box background. Next, a mask was made to eliminate shadows caused by the slit cut for the stem and the edge of the filter paper. The erosion operation was used to remove a 5-pixel-wide strip from the circumference of the filter paper. This procedure was used to exclude similar shadows on all images of stained filter paper.

The threshold operation was used again to isolate the stained area from the rest of the filter-paper image. The software was used to create 254 levels of a gray scale to differentiate stain intensity. Levels 1 – 88 were unstained filter paper, and only levels 89 through 254 were used in each analysis. A histogram of the image was generated, and the results were stored in a digital data table. The data tables resulting from this image analysis procedure consisted of gray levels (89 – 254) and their corresponding pixel count. The stain on each filter paper was quantified by summing each gray level multiplied by its corresponding pixel count. This integration procedure accounted for honeydew droplets that were overlapping and otherwise may have resulted in underestimates of feeding.

Two separate analyses were run on the quantity of stained honeydew results. One used the raw data and the other used values adjusted for mortality. The analysis on unadjusted measures provided greater precision over the analysis using adjusted measures. Therefore, only results of the unadjusted measures are presented. Analysis of variance was used to compare differences in honeydew among treatments (SAS 1990). Means were separated using Fishers least significant difference ($P = 0.05$) test when the ANOVA was significant.

Results and Discussion

Data were analyzed by experiment because the statistical analysis showed significant differences among experiments ($F = 95.08$; $df = 2, 5$; $P < 0.01$). These differences were likely caused by variable lighting conditions when photographs from each trial were taken. All three experiments resulted in acceptable levels of precision. The coefficient of variation was 32.6 for the first trial using first regrowth of greenhouse-grown clones, 60.7 for the fourth regrowth of greenhouse grown clones, and 46.0 for the field-grown clones.

There was no evidence that adult leafhoppers fed more or less on the susceptible control, compared to any level of leafhopper-resistant treatments (Table 1). Significant differences among treatments were found only in the trial using the first regrowth of greenhouse-grown alfalfa ($F = 3.44$; $df = 5, 35$; $P < 0.05$). However, there was no trend for more or less feeding as the level of resistance increased. There were no differences in quantity of honeydew among treatments in the trials using fourth regrowth of greenhouse-grown clones ($F = 2.41$; $df = 5, 35$; $P > 0.05$) and field grown clones ($F = 0.28$; $df = 5, 35$; $P > 0.05$).

These results show that leafhoppers were capable of feeding heavily on any level of glandular-haired 5347LH alfalfa, compared to susceptible alfalfa. The same conclusion could not be reached if hopperburn had been used to estimate feeding on clones resistant to leaf yellowing. Leafhopper feeding would be underestimated in this instance. Indeed, there is strong evidence that 5347LH shows less hopperburn than 645 under the same leafhopper pressure (S.A.L. and L.P.P., unpublished data).

It is imperative that entomologists and alfalfa breeders alike do not confuse a lack of hopperburn with a lack of leafhopper feeding. This potential error could confound the ability to decipher resistance mechanisms and degrade the effectiveness of selection criteria in alfalfa breeding programs. Understanding the quantity of insect feeding is imperative in host plant resistance research, and we propose this technique as a simple and precise alternative for the potato leafhopper and possibly other assimilate-feeding insects.

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Table 1. Mean and standard error of quantity of adult potato leafhopper feeding on alfalfa.

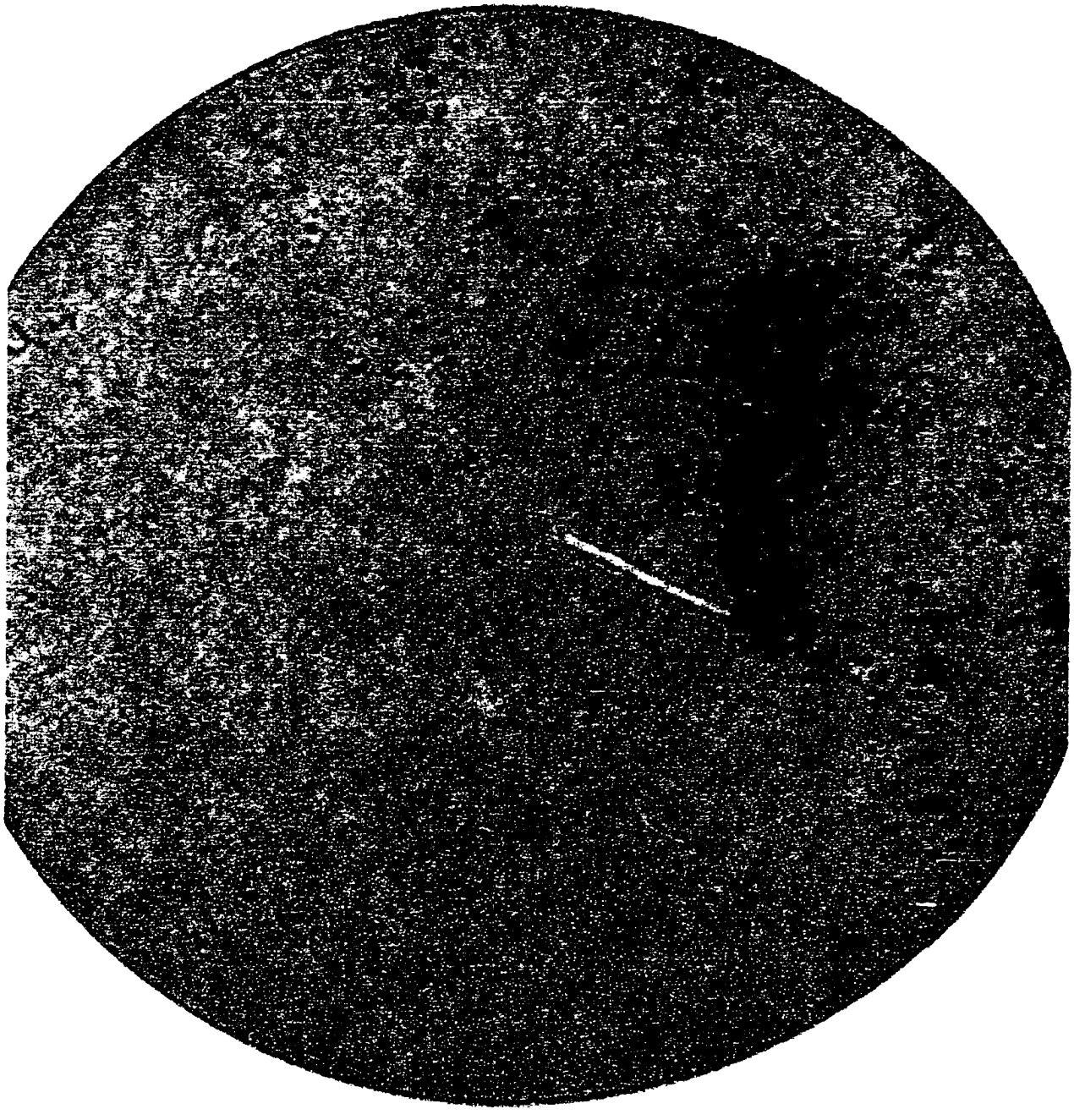
Alfalfa clone[a]	Quantity of honeydew[b]
	First regrowth, greenhouse grown clones
S	$773x10^3 \pm 101x10^3$abc
1	$638x10^3 \pm 59x10^3$bc
3	$979x10^3 \pm 97x10^3$a
5	$808x10^3 \pm 64x10^3$ab
7	$629x10^3 \pm 71x10^3$bc
9	$550x10^3 \pm 94x10^3$c
	Fourth regrowth, greenhouse grown clones
S	$184x10^3 \pm 40x10^3$
1	$120x10^3 \pm 27x10^3$
3	$246x10^3 \pm 67x10^3$
5	$186x10^3 \pm 44x10^3$
7	$158x10^3 \pm 40x10^3$
9	$91x10^3 \pm 20x10^3$
	Spring regrowth, field grown clones
S	$1103x10^3 \pm 245x10^3$
1	$977x10^3 \pm 74x10^3$
3	$866x10^3 \pm 137x10^3$
5	$967x10^3 \pm 168x10^3$
7	$1002x10^3 \pm 135x10^3$
9	$897x10^3 \pm 95x10^3$

Means sharing the same letter are not significantly different (P = 0.05).

[a] S, susceptible; 1, least resistant; 9, most resistant.

[b] Values equal the result of integrating gray scale level and pixel count.

Figure 1. A honeydew-stained filter paper that shows how droplets vary in size and intensity and that they may overlap.



CHAPTER 4. POTATO LEAFHOPPER ANTIXENOSIS IN A LEAFHOPPER-RESISTANT ALFALFA VARIETY

A paper submitted to the Journal of Economic Entomology

Stephen A. Lefko, Larry P. Pedigo, and Marlin E. Rice

Abstract

Free-choice and no-choice tests were used to investigate the effect of alfalfa resistance on the feeding behavior of the potato leafhopper *Empoasca fabae* (Harris). Quantity of feeding and feeding preference was compared among a susceptible clone and 5 clones representing a range of resistance levels in 5347LH® alfalfa. Both experiments used 8 replications per trial. Three trials were run using the initial and 4th growth intervals of greenhouse-grown clones, and once on the spring regrowth of one-year-old, field-grown clones. In the free-choice tests, the treatments consisted of 6 different stem pairs: one pair of susceptible stems, and 5 pairs where a susceptible stem was paired with one stem of each level of resistance. Three unsexed adult leafhoppers were caged for 7 days on stem pairs and a 1–5 index was used to score injury. Injury to resistant stems decreased significantly as more resistant stems were substituted in cages. Conversely, injury increased significantly on the susceptible stems as more resistant stems were substituted in cages. This strongly suggests a stem-level antixenotic resistance mechanism, which increased in efficacy as the alternate host increased in resistance. In the free-choice tests, ten adult leafhoppers were caged on single stem cuttings for 24 h, and honeydew was collected on filter paper disks at the base of each cage. The 6 treatments were the susceptible clone and the 5 levels of resistance from 5347LH. The quantity of honeydew was measured using a staining and image analysis technique. Adult survival was also measured in cages. Results showed no

difference in feeding or adult survival among the 6 treatments. The efficacy of this antixenotic mechanism appeared dependent on insect behavior and the availability of alternate hosts, because the leafhoppers could feed on resistant clones.

Introduction

The potato leafhopper, *Empoasca fabae* (Harris), is a key pest of alfalfa (*Medicago sativa* L.) in the upper Midwest and northeastern United States. Plant stunting and leaf yellowing, or hopperburn, are 2 symptoms of potato leafhopper feeding on alfalfa. These symptoms usually occur together and, although hopperburn is the most visible symptom, economic loss is most highly correlated with plant stunting (Hutchins et al. 1989).

Ecale and Backus (1995a) showed that symptoms of potato leafhopper in alfalfa result more from the deposition of salivary enzymes compared with the mechanical damage of the vascular tissues during feeding. Moreover, Ecale and Backus (1995b) showed how leafhopper feeding caused phloem cells to collapse and xylem cells to shrink, and they described how these changes alone could cause the symptoms of wilting, hopperburn, and stunting and, ultimately, reduce alfalfa yield.

Taylor (1956) showed that pubescence was a heritable trait in alfalfa that could be exploited for developing resistance to potato leafhopper. More recent studies have investigated the mechanism of resistance in perennial glandular-haired alfalfa and highlighted the role of pubescence. Brewer et al. (1986) used free-choice tests to investigate feeding antixenosis among 5 glandular-haired clones with dense pubescence and 2 susceptible controls. They found less injury on most of the glandular-haired clones compared with the susceptible control. One hypothesis used to describe antibiosis was reduced feeding because of a tough vascular cylinder. Elden and McCaslin (1997) showed

significant but not strong correlations between the density of glandular hairs and resistance to potato leafhopper in 19 clones of glandular-haired alfalfa. They also stated that glandular hairs on these perennial glandular-haired clones did not entrap leafhoppers, and suggested that an unexplained resistance mechanism may exist. Similarly, Elden and Elgin (1992) found that leafhopper injury was often worse on susceptible alfalfa when caged with 6 other clones that had dense simple hairs. Combined, these studies clearly show the presence and, possibly, the predominance of an antixenotic mechanism, and they tend to link antixenosis with pubescence. In all these instances pubescence (glandular or not) could have been a physical barrier and reduced the insect's ability to feed as usual.

A stand of resistant alfalfa is a heterogeneous population of plants (McCoy and Bingham 1988), and Othman et al. (1981) and Elden and McCaslin (1997) showed how the density and distribution of glandular hairs can differ among alfalfa lines and plants within lines. Therefore, the availability and level of resistance found in alternate hosts likely will influence the efficacy of this mechanism in the field.

The objectives of this study were to (1) investigate the presence of a stem-level antixenotic mechanism in a leafhopper-resistant alfalfa, (2) determine how alternative hosts with variable levels of antixenosis influence injury, and (3) determine if dense pubescence impedes adult feeding.

Materials and Methods

Five individual alfalfa plants were selected from a field population of glandular-haired 5347LH® alfalfa that was repeatedly stressed by naturally occurring populations of potato leafhopper. These plants were selected based on a 1–9 hopperburn rating scale. This scale used the same injury criteria as the standard test (1–5) for potato leafhopper resistance

(McCaslin and Miller 1996), except it was inverted (1, severe injury, 9, no apparent injury) and provided more intermediate ratings. The 5 glandular-haired clones used in the study represented resistance levels 1, 3, 5, 7, and 9. An additional plant from a leafhopper-susceptible population of 645® variety was selected, at random, for the control. Each plant was cloned from stem cuttings for use as replicates in the study. Individuals, representing the 6 clones, were maintained in the greenhouse under a 16:8 photoperiod and 25:18°C temperature regime. Also, 10 cuttings of each clone were transplanted to the field during the summer of 1997. All plants were cut when they reached the late-bloom stage.

Cages used for free-choice and no-choice experiments were fashioned from inverted 0.47 liter (1-pint) ice-cream containers. Each cage had 2 0.5-cm holes cut through the lid, so the alfalfa stems could pass through and reach a water source below (Fig. 1). These holes were covered with latex, and a slit was cut to allow the stem to pass through but to prevent leafhoppers from leaving the cage. Water was held in another small plastic cup with a lid that was glued to the lid of the ice-cream container. The removable cage-top, which was the base of the ice-cream container, had the bottom cut out and replaced with fabric screen.

Free-choice and no-choice experiments each were run 3 times, once on the 1st regrowth of greenhouse-grown clones, once on the 4th regrowth of greenhouse-grown clones, and once on the spring 1998 regrowth of field-grown clones. These field-grown clones grew to harvest maturity twice during 1997, before winter. For both experiments, cages were placed in an environmental chamber, arranged as a randomized complete block, with 8 replications of each treatment. The photoperiod and temperature regime were 16:8 (L:D) and 25:18°C, respectively.

Greenhouse colonies of potato leafhopper were reared on broad bean (*Vicia faba* L.) using a photoperiod of 16:8 (L:D) and a corresponding temperature regime of 25:18°C. Colonies were reinfested with field-collected adults each summer.

Free-Choice Experiment

The 6 treatments used were 6 pairs of alfalfa stems. These treatments were: 1 pair of susceptible stems and a susceptible stem paired with each of the 5 resistance levels of 5347LH. Each stem pair was caged and held in the environmental chamber for 24h before infestation. This holding period allowed stems that might have been damaged during cutting to wilt and be replaced, if necessary. Stem cuttings were taken from below the last node with a fully expanded leaf. Each cutting had 3 nodes and was positioned with 1 node below and 2 above the filter paper.

Cages were infested with 3 unsexed adult potato leafhoppers collected from the greenhouse colonies. Leafhoppers were allowed to feed for 7 days, then a 1–5 index was used to rate injury to both stems in each cage. The index categories were (1) no injury, (2) 1/3 leaves showing hopperburn, (3) 2/3 leaves showing hopperburn, (4) all leaves showing hopperburn, and (5) all leaves showing hopperburn and some necrosis.

Linear regression was used to determine if injury increased on resistant and susceptible stems as more resistant stems were substituted in cages (SAS 1990). This procedure was repeated for all 3 trials, and comparisons of slopes were made among trials according to alfalfa stem-type (susceptible or resistant) (Zar 1984).

No-Choice Experiment

The quantity of leafhopper feeding was measured indirectly by estimating the quantity of honeydew produced. Treatments included the susceptible stem and the 5 levels

of 5347LH resistance. Treatments were replicated 8 times in each trial and stem cuttings were prepared similar to those used in the free-choice study. Cages were infested after 24h with 10 unsexed adult leafhoppers per cage from the same greenhouse colonies. Preliminary experiments showed that 10 leafhoppers produced enough honeydew in 24 h to complete the analysis.

After the 24 h feeding interval, surviving adults were counted, and the filter paper was removed. Honeydew collected on filter paper disks was stained using 0.2% ninhydrin in ethanol (Sigma, St. Louis, MO) and heated immediately in a 100°C oven for 10 minutes. Digital images of each filter paper were taken and the quantity of stained honeydew was estimated using an image analysis technique outlined by Lefko et al. (in press b). Histograms of pixel color intensity and pixel count were generated, and the stain was quantified for each disk by integrating color intensity and pixel count. Analysis of variance was used to compare differences in honeydew and survival among treatments (SAS 1990). Means were separated using Fishers Least Significant Difference ($P = 0.05$) test if the ANOVA was significant.

Results and Discussion

Free-Choice Experiment

Regression analysis showed a significant decrease in leafhopper injury on resistant stems as stems with greater resistance were substituted in cages (Table 1). Slopes resulting from each trial were not statistically different ($P = 0.05$); therefore, data were pooled and a new linear model was fitted. Pooled data showed a similar and significant decrease in injury as stems with greater resistance were substituted in each cage ($F = 40.0$; $df = 1, 142$; $P < 0.01$) (Fig. 2).

The decline in injury on stems with increasing resistance could have been caused by increased resistance to yellowing (Kindler and Kehr 1973; Jarvis and Kehr 1966) or a feeding preference for the susceptible stems. Therefore, injury to resistant stems cannot be used alone to show antixenosis because these 2 possibilities cannot be differentiated.

A stem-level antixenotic mechanism is likely the best explanation for the injury seen on susceptible stems. Injury increased on susceptible stems in each trial as stems with greater resistance were substituted in each cage (Table 1). Again, slopes resulting from each trial were not statistically different; therefore, data were pooled and a new linear model was fitted (Fig. 2). The regression using pooled data from susceptible stems showed a significant increase in injury as stems with greater resistance were substituted in cages ($F = 6.6$; $df = 1$, 142; $P < 0.05$). Other studies using glandular-haired alfalfa and alfalfa with dense pubescence corroborate these findings (Brewer et al. 1986; Elden and Elgin 1992).

Adult Survival

Aspirating likely caused some physical injury to leafhoppers used in the experiment. But, the average number of leafhoppers surviving the 24 h feeding period never was less than 8.25, out of a possible 10.0, for any treatment. Moreover, leafhopper survival did not differ among treatments in any of the 3 trials, and there was no tendency for reduced survival in any of the resistant treatments (Table 2).

This was not a true test for an antibiotic resistance mechanism in leafhopper-resistant alfalfa because of the short duration and lack of stage-specific data. Others have shown, however, that some lines of glandular-haired alfalfa have an antibiotic mechanism (Brewer et al. 1986; Elden and McCaslin 1997), even though it remains un-described. Results of our study suggest the absence of a mechanism capable of quickly killing adults (24h). Some of

these quick-kill mechanisms could be physical entanglement (Shade et al. 1979), an alfalfa morphological structure that prevents adequate feeding (Brewer et al. 1986; Elden and Lambert 1992), or trichomes that secrete toxic substances.

Quantifying Stained Honeydew

The statistical analysis showed significant differences among the 3 no-choice trials, which precluded pooling all 3 data sets ($F = 95.08$, $df = 2, 5$, $P < 0.01$). All three trials resulted in favorable levels of precision. The coefficient of variation was 32.6 for the 1st trial, using first regrowth of greenhouse-grown clones, 60.7 for the 4th regrowth of greenhouse-grown clones, and 46.0 for the field-grown clones. One explanation for the differences among trials may be the calibration process of the image analysis equipment before each trial (Lefko et al. in press b).

There was no evidence that adult leafhoppers fed more or less on the susceptible control compared with any level of leafhopper-resistant treatments (Table 2). Significant differences among treatments were found only when the 1st regrowth of greenhouse-grown alfalfa was used ($F = 3.44$, $df = 5, 35$, $P < 0.05$). However, there was no trend for more or less feeding as the level of resistance increased. There were no differences in quantity of honeydew among treatments in the trials using 4th regrowth of greenhouse-grown clones ($F = 2.41$, $df = 5, 35$, $P > 0.05$) and field-grown clones ($F = 0.28$, $df = 5, 35$, $P > 0.05$).

These results show that leafhoppers were capable of feeding heavily on any level of resistance in the 5347LH glandular-haired alfalfa compared with susceptible alfalfa. Moreover, it seemed that the morphological structure of glandular-haired alfalfa did not impede leafhopper feeding compared with susceptible alfalfa during a 24h period. The same conclusion could not be reached if hopperburn was used to estimate feeding, and clones were

resistant to leaf yellowing. Leafhopper feeding would be underestimated in this instance. Indeed, there is strong evidence that 5347LH resists hopperburn (Lefko and Pedigo, unpublished data).

Conclusion

These results show that a potato leafhopper antixenotic mechanism functions among resistant and susceptible alfalfa stems. Moreover, the efficacy of this mechanism increases as the difference between the levels of resistance of available hosts becomes greater. The no-choice study showed that without an alternate host, leafhoppers fed as much on the levels of 5347LH resistance used in this study compared with leafhopper-susceptible alfalfa. Therefore, it seems the efficacy of this antixenotic mechanism is largely a function of insect behavior and the level of resistance and proximity of alternate hosts. It is important to mention the presence of this mechanism does not exclude the potential presence of other resistance mechanisms, namely antibiosis and tolerance (Painter 1951). These findings, coupled with results from earlier studies, are useful for predicting potato leafhopper response to alfalfa resistance, as it occurs in production.

Hogg et al. (1998) and Lefko et al. (in press a) conducted comparative studies of adult and nymphal leafhopper population size in field plots of glandular-haired and susceptible alfalfa, including 5347LH. Both reports showed the adult population density was similar between resistant alfalfa and the control(s). Although Hogg et al. (1998) found fewer nymphs in glandular-haired alfalfa, Lefko et al. (in press a) did not find these differences. Moreover, Lefko et al. (in press c) found that nymphal production was not different between several varieties of glandular-haired alfalfa and a susceptible control when leafhoppers were caged on a small stand. One explanation for these similarities between resistant and

susceptible alfalfa may result from a preference for less resistant stems in a heterogeneous stand. Leafhoppers may aggregate on the proportion of less resistant plants, and results from this study show that adult feeding is equally possible on these plants. Therefore, the carrying capacity of 5347LH may still be great enough that the leafhopper population is similar in size to one found in susceptible stands of alfalfa (Lefko et al. in press c).

Acknowledgments

We greatly appreciate the help of Clive Holland, Gary Hoard, and Tim Woodward of Pioneer Hi-Bred Int. Inc. for supplying the 5347LH alfalfa clones. Many thanks go to Mike Nagel, Eng Han Low, Kendra Dvorak, and Robert Yaklich for maintaining the insect colonies and alfalfa clones. Funding for this research was provided in part by a competitive grant from Pioneer Hi-Bred Int. Inc. This is Journal Paper No. J-18206 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 3207.

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Table 1. Slopes of regression analyses relating injury to alfalfa type as treatments increased in resistance.

	Alfalfa stem type[a]	
Alfalfa age	Resistant	Susceptible
Initial growth	-0.254 ± 0.045**	0.186 ± 0.104
Fourth regrowth	-0.275 ± 0.071*	0.211 ± 0.100*
Spring regrowth	-0.143 ± 0.060*	0.032 ± 0.076

[a] Slope different from zero; *, $P = 0.05$; **, $P = 0.01$

Table 2. Mean and standard error of quantity of adult potato leafhopper feeding and survival on alfalfa.

Alfalfa clone[a]	Quantity of honeydew[b]	Survival[c]
	First regrowth, greenhouse grown clones	
S	$773x10^3 \pm 101x10^3$abc	9.13 +- 0.40
1	$638x10^3 \pm 59x10^3$bc	8.50 +- 0.33
3	$979x10^3 \pm 97x10^3$a	9.25 +- 0.31
5	$808x10^3 \pm 64x10^3$ab	9.25 +- 0.25
7	$629x10^3 \pm 71x10^3$bc	8.88 +- 0.52
9	$550x10^3 \pm 94x10^3$c	8.50 +- 0.53
	Fourth regrowth, greenhouse grown clones	
S	$184x10^3 \pm 40x10^3$	8.50 +- 0.53
1	$120x10^3 \pm 27x10^3$	9.38 +- 0.26
3	$246x10^3 \pm 67x10^3$	8.25 +- 0.77
5	$186x10^3 \pm 44x10^3$	8.88 +- 0.58
7	$158x10^3 \pm 40x10^3$	9.38 +- 0.26
9	$91x10^3 \pm 20x10^3$	8.75 +- 0.56
	Spring regrowth, field grown clones	
S	$1103x10^3 \pm 245x10^3$	8.75 +- 0.31
1	$977x10^3 \pm 74x10^3$	8.63 +- 0.37
3	$866x10^3 \pm 137x10^3$	9.13 +- 0.40
5	$967x10^3 \pm 168x10^3$	8.38 +- 0.33
7	$1002x10^3 \pm 135x10^3$	8.88 +- 0.40
9	$897x10^3 \pm 95x10^3$	8.25 +- 0.53

Means in column categories sharing the same letter are not significantly different ($P > 0.05$).

[a] S, susceptible, 1, least resistant, 9, most resistant.

[b] Values equal result of integrating the number of pixels per color using image analysis.

[c] Values equal the number out of 10 adults that survived.

Figure 1. Cage type used in both feeding experiments.

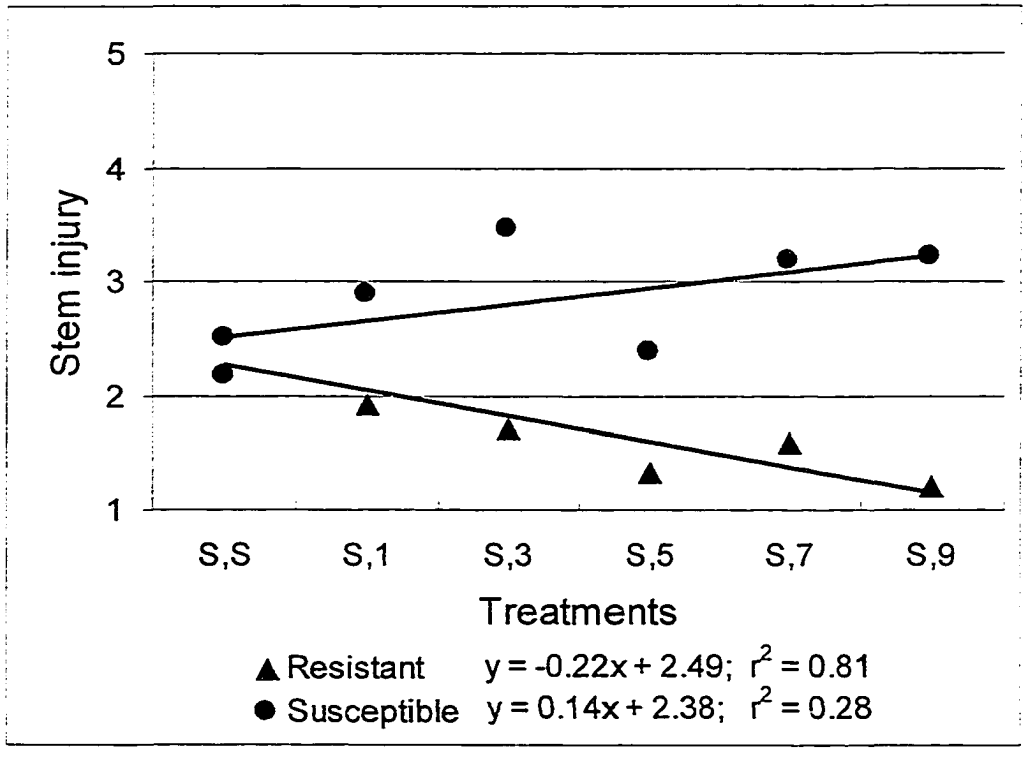

Figure 2. Linear regressions of average injury to alfalfa stem types as more resistant stems were substituted in treatments. Treatments: S,S pair of susceptible stems; S, 1 susceptible paired with least resistant clone; S,9 susceptible paired with most resistant clone.

CHAPTER 5. ALFALFA STAND-TOLERANCE TO POTATO LEAFHOPPER AND ITS EFFECT ON THE ECONOMIC INJURY LEVEL

A paper submitted to the Journal of Economic Entomology

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Abstract

In 1997 several seed companies released alfalfa products that were marketed as resistant to the potato leafhopper (*Empoasca fabae* Harris), the key pest of this crop in the Midwest and northeastern USA. The objectives of this research were to investigate the mechanism of insect resistance and determine if potato leafhopper-resistant alfalfa would require updated pest management guidelines. Two experiments were planted in Ames, IA. Four (first generation) resistant alfalfas were compared with a susceptible control in the experiment planted in 1996. Another experiment was planted in 1998 to compare the same control with three other (second generation) resistant alfalfas. Four cages were used in each plot to create four levels of leafhopper stress on alfalfa. Cages were infested with adult leafhoppers, and nymphs were collected from inside cages when the alfalfa was harvested. Estimates of alfalfa dry weight were used to calculate linear yield–loss models, and model coefficients were used to calculate economic injury levels and economic thresholds. Trials were run on seedling, second-cutting seeding-year, second-cutting second-year, and second-cutting third-year alfalfa. There were no measurable differences in nymph production on resistant or susceptible alfalfa in any trial, which indicated that an antibiotic mechanism was seemingly unimportant under production conditions. The potential for resistant alfalfa to outperform susceptible alfalfa under leafhopper stress began after the initial growth of seedlings and continued through year three. The mechanism was described as stand-

tolerance and seemed to increase as the alfalfa stand matured. The onset of stand-tolerance raised the economic threshold from 8 to 80 leafhoppers per 10 sweeps. The potential for loss from potato leafhopper decreased greatly in susceptible alfalfa from the first to the second year of the stand, and this could greatly increase the economic threshold.

Introduction

One of the most recent commercial advancements in alfalfa (*Medicago* spp.) pest management targets a key pest in the Midwest and Northeast, the potato leafhopper, *Empoasca fabae* (Harris). In 1997 several seed companies released alfalfa varieties that were advertised as resistant to the potato leafhopper. This ubiquitous pest is an assimilate remover that causes stunting and leaf chlorosis in alfalfa and other crops. Economic loss from potato leafhopper is best linked to reductions in alfalfa biomass and less so of forage quality (Hutchins et al. 1989a).

The potential for leafhopper-resistant alfalfa was first recognized in the 1980's when Sorensen et al. (1985, 1986) released perennial alfalfa with multiple pest resistance. Releases were referred to as glandular-haired alfalfa. Use of the term glandular-haired alfalfa may be a relic from the work of Shade et al. (1979) who showed that hairs on an annual alfalfa secreted a sticky substance that entangled small insects.

Researchers have investigated the presence and importance of antibiosis, nonpreference, and tolerance in perennial resistant alfalfa. Most studies have emphasized the importance of pubescence in conferring resistance. Brewer et. al. (1986a) compared three species of resistant (glandular-haired) alfalfa to one susceptible hay type. Mortality was greatest on the resistant alfalfa, compared with the control, when there was no choice. However, most of the clones of resistant alfalfa showed feeding and ovipositional

nonpreference when a susceptible host was available. The authors did not detect an entrapment mechanism similar to the one described by Shade et al. (1979) in annual glandular-haired types. In a separate study, Brewer et al. (1986b) described how highly lignified tissues might enhance resistance to the leafhopper.

Elden and Elgin (1992) also performed free-choice and no-choice experiments on alfalfa having dense pubescence and resistance to multiple pests. They concluded that some clones had high levels of feeding and ovipositional nonpreference and nymphal antibiosis. Similarly, clones that were antibiotic in no-choice tests seemed to have a nonpreference mechanism when alternate hosts were available. Their conclusions on nymphal and ovipositional nonpreference should be interpreted with caution. It seems that the effect of adult (female) mortality and, consequently, total oviposition were unaccounted for and could be the cause of the variation in the number of nymphs produced on each line of alfalfa.

Elden and McCaslin (1997) conducted no-choice studies and showed a significant but weak correlation between the density of glandular hairs and resistance to potato leafhopper in 19 glandular-haired alfalfa clones. Estimates of nymphal mortality ranged from 0 to 33%; however, it seems nymphal mortality was not corrected for adult (female) survival, which likely influenced total oviposition. They reported 13%–96% mortality of adult leafhoppers in no-choice cage tests. They also stated that glandular hairs on perennial clones did not entrap leafhoppers and suggested that an unexplained resistance mechanism may exist. These studies using stem cuttings have been paramount to a better understanding of the mechanism of resistance. It is difficult, however, to extrapolate their results to field conditions without great uncertainty. Using these results, it seems producers could expect

suppressed leafhopper numbers; antibiosis would be overridden by nonpreference since alternate hosts would be available (Poos and Wheeler 1943).

Hogg et al. (1998) and Lefko et al. (1999) conducted comparative studies of adult and nymphal population size in field plots of glandular-haired and susceptible alfalfa. This kind of study could be used only to reject the presence of a plot-level nonpreference mechanism or implicate, but not differentiate, between antibiosis and nonpreference. Both reports showed the adult population density was similar between resistant alfalfa and the control(s) in both studies. Hogg et al. (1998) found fewer nymphs in glandular-haired alfalfa, however, Lefko et al. (1999) did not find these differences. Both studies concluded that nonpreference is an unlikely explanation for the resistance mechanism at a production scale.

While antibiosis and nonpreference have been the focus of most studies, tolerance has received little attention. This mechanism seems important because Hogg et al. (1998) and Lefko et al. (1997) showed the population density of potato leafhopper was similar between resistant and susceptible alfalfa, yet there was a yield advantage from resistant alfalfa when the leafhopper densities were high.

The objective of this research was to determine if the alfalfa yield response to potato leafhopper was different for resistant and susceptible alfalfa and if the potato leafhopper population growth-potential was different among small stands of resistant and susceptible alfalfa. Results would help determine if tolerance is an important mechanism, and if so, how pest management guidelines could accommodate it.

Materials and Methods

Experiments used to calculate economic injury levels and investigate the mechanisms of resistance were conducted in the field during 1996, 1997, and 1998. One experiment was

planted on 17 April 1996 at the Iowa State University Johnson Research Farm near Ames, IA. The treatments were three commercial resistant varieties (AmeriGuard 301®, Trailblazer®, and 5347LH®), an experimental resistant alfalfa (XAE49), and a susceptible control (645®) that had historically high yields in Iowa. These three resistant commercial varieties were marketed as the first generation of potato leafhopper-resistant alfalfa. Treatments were planted in plots according to a randomized complete block design using four replications. Seed was planted using a single-row hand planter. Rows were spaced 19 cm apart and the planter was calibrated to 11.12 kg/ha (10lb/acre). Seed availability limited individual plot size to 1.5 m by 3.7 m. Plots were located in a newly seeded 0.6 ha field of susceptible alfalfa.

A second experiment was initiated on 27 April 1998 in a neighboring field at the same farm. Resistant alfalfas used in the experiment were 53V63, 54H69, and 3A09. The susceptible control again was 645. Of the resistant varieties, 53V63 and 54H69 are not yet commercial as of 1998. These two resistant varieties have been designated second-generation products and likely have greater resistance to potato leafhopper. The 3A09 alfalfa is an experimental line. These plots measured 1.5 m by 7.4 m and were arranged according to a randomized complete block design, also using four replicates. This experiment was planted with a culter-packer style cone planter calibrated to deliver seed at 16.67 kg/ha (15 lb/acre).

All alfalfa was managed by using a three cut system with two cuts during the seeding year. Alfalfa was harvested when it reached the early to mid-bloom stage.

Potato leafhoppers were caged on alfalfa to achieve different levels of pest pressure. Cages were constructed from plastic refuse containers that measured 70-cm tall by 52-cm

diameter at the open end (Fig 1). The opening covered a land area of $0.21m^2$. Side panels and the bottom were cut from the containers and 32x32 Lumite® mesh (Synthetic Industries, Inc., Gainesville, GA) was attached in their place. Containers were reinforced on the inside with wooden lath and secured to the ground using tent stakes.

Trials began by treating freshly cut or seedling alfalfa with an insecticide (Pounce 3.2 EC) to kill the existing insect population. Cages were placed over alfalfa that was uniform in plant density immediately after the treatment.

Cages were infested with adult leafhoppers after the alfalfa had reached a height of 7 cm to 10 cm. Leafhoppers were caged at four levels in each plot, and levels were randomly assigned to cages before each trial. Leafhopper levels were increased after the seeding year (Table 1). The increases were necessary to produce a greater alfalfa yield response as the plants aged. Leafhoppers were reared in greenhouse colonies on broad bean (*Vicia faba* L.), by using a photoperiod of 16:8 (L:D) and a temperature regime of 25:18°C. These colonies were reinfested with field-collected adults each summer. The percentage of adult females in the populations varied from 56%–70% among trials.

Economic injury level trials were performed on four separate occasions. Three trials were conducted on the first-generation alfalfa and one on the second–generation alfalfa. The year, growth interval, and infestation levels used in each trial are summarized in Table 1.

Each trial ended by collecting the plant material and nymphs from each cage. Nymphs were collected by cutting the alfalfa inside the cage then submerging and agitating the alfalfa in 70% ethanol plus 30% water for 30 seconds. Afterwards, nymphs were filtered from the ethanol and counted, and the alfalfa was bagged and dried at 60°C for 72h. Dry matter of alfalfa from each cage was weighed immediately after the drying period.

Nymph counts per cage were converted to an index of population growth to normalize values across all levels of infestation. The conversion equation was:

$$nymph\_index = \frac{n_f - x_o}{n_i}$$

where x_o equals the mean number of nymphs from replications with the zero level of infestation, n_i equals the number of adults infested, and n_f equals the number of nymphs collected within a cage at harvest. Subtracting x_o accounted for nymphs or eggs that were not killed by the insecticide. Values of the index greater than one indicate a nymph density larger than the initial density of adults. Of the 16 cages per treatment, only 12 were used in the analysis since the four cages not infested were used to estimate x_o for each treatment. An analysis of variance (SAS 1990) was used to determine if the average index value was different among varieties for each trial.

Alfalfa dry-weight and leafhopper density data were used to calculate yield loss equations for each variety in each trial. Estimates of percentage loss were calculated for infested cages using the zero level of infestation in each plot as a basis. Least squares linear regression (SAS 1990) was used to calculate linear model coefficients. Linear models were fit to average percentage loss values for each level of infestation according to treatment. Models were recalculated and forced through the origin if the original Y intercept was positive. This procedure was used to maintain biological meaning at the expense of statistical significance (r^2), since a positive Y intercept indicates yield loss from the potato leafhopper when its number is zero. Differences between slopes and intercepts of alfalfa were tested in each trial using a Student's t-test performed on all pair-wise combinations of

alfalfa (Zar 1984). The same procedure was used to test differences among slopes for 645, the susceptible control, among trials.

Economic injury levels were calculated using the equation:

$$EIL = \left(\frac{100C}{VY_p} + a \right) \times \frac{1}{b} \times \frac{1}{K}$$

where C = cost of treatment per acre; V = value of alfalfa per acre; Y_p = potential alfalfa yield in tons per acre per cutting; a = y intercept of yield loss equation; b = slope of the yield loss equation, and K = proportion reduction in potential injury or damage. This equation is modeled after equations described by Pedigo (1999). Economic parameters were an $8 cost of treatment and a crop value of $70 per ton and were held constant for all calculations of economic injury level. The expected alfalfa yield used in all equations was 680 kg/ha/cutting (1.67 tons/acre/cutting). Absolute densities calculated from this equation were converted for use with a relative sampling technique. DeGooyer et al. (1998) published regression equations for converting the absolute estimate per $0.25m^2$ to an estimate found using 10 sweeps with a sweepnet ($Y = 1.27X + 5.07$; $r^2 = 0.82$). The economic threshold was calculated by reducing the economic injury level by 25%.

Results

Index of population growth

There was no evidence that resistant (glandular-haired) alfalfa had a negative effect on leafhopper population-growth when caged on small plots of alfalfa. Most index values were greater than 1.0, which indicated more nymphs were collected than adults infested (Table 1). Values ranged from 0.14 ± 0.40 to 3.61 ± 0.27 for resistant alfalfa, and 0.22 ± 0.60 to 3.71 ± 0.51 for the susceptible control. The largest difference between average index

values in one trial was between 645 and 53V63 in 1998, and the index from 53V63 was significantly larger, compared to 645 ($F = 3.38$; $df = 3, 9$; $P < 0.10$). This difference is likely a result of experimental error and not biologically important.

Index values less than one likely resulted from poor leafhopper control in cages before the trial, which resulted in nymph populations in uninfested cages. High numbers would decrease the numerator in the conversion equation and result in smaller index values.

Yield loss coefficients

Comparisons of linear model coefficients were made for all pair-wise combinations of alfalfas. Also, coefficients were compared among years for both alfalfa types. The slope of each model equals the percentage of yield loss per $0.21 m^2$ expected for each additional potato leafhopper. This coefficient is referred to as the loss rate throughout this paper. The loss rate is one variable used to calculate the economic injury level, and lower values result in higher economic thresholds. Another important coefficient is the intercept of the linear model. The x-axis intercept is the pest number when loss begins.

There was a trend for lower loss rates and positive x-axis intercepts in resistant alfalfa compared with the susceptible control. However, these differences were only detected in regrowth intervals after the initial growth of seedling alfalfa. Additionally, there was a trend for loss rates of all alfalfa to decrease as stands aged. Results in the following paragraphs are reported beginning with the trial performed on the youngest alfalfa and ending with the trial on the oldest alfalfa.

The trial performed on the initial (seedling) growth of resistant alfalfa showed no trend for smaller loss rates in resistant alfalfas, compared to the susceptible control (Table 1). The only statistical difference between loss rates was found between 53V63 and 645 ($t =$

2.83; $df = 4$; $P = 0.05$), and 53V63 and 54H69 ($t = 3.14$; $df = 4$; $P = 0.05$). These differences are unimportant. Large experimental error probably caused the negative, and consequently meaningless, loss rate for 53V63. Both the control and 3A09 had positive X-intercepts, however there was no statistical difference between these values.

The onset of resistance was first detected in the next trial that used the second cutting of a seeding-year stand. All loss rates for resistant alfalfas were less than the control in this trial (Table 1). The loss rates of AmeriGuard 301 and 5347LH were significantly lower than the control ($t = 5.49$; $df = 4$; $P = 0.05$, $t = 5.61$; $df = 4$; $P = 0.05$). Therefore, these resistant varieties had a significant yield advantage over the control at this age and within this range of pest pressure. Of the resistant varieties, Trailblazer had the highest loss rate (0.158); however, this loss rate was not different from all other resistant alfalfas. Conversely, the regression fit to Trailblazer data had the highest x-axis intercept (38.8), although it was not statistically different from the others. Even though the yield advantage of resistant alfalfa was detected at this plant age, the mechanism of resistance was not obvious because resistant alfalfa had no measurable effect on nymphal production.

Levels of leafhopper infestation were doubled for resistant alfalfa compared with susceptible alfalfa in the trial using the second cutting of second-year alfalfa (Table 1). This adjustment was necessary because of the relatively low loss rates in the previous trial. Surprisingly, the yield response was less evident in this trial, compared with the previous trial. Statistically, there were no differences in loss rates among alfalfas. The loss rate of the control remained much higher. It was more than four times larger than the lowest loss rate of resistant alfalfas even though twice as many leafhoppers were caged on resistant alfalfas. Only 5347LH had a positive x-axis intercept. Even though a yield advantage persisted in

leafhopper-resistant alfalfa, an effect of plant resistance on leafhopper population growth was absent (Table 1).

Levels of infestation were increased again in the next trial using second-cutting third-year alfalfa. The control infestation levels were 0, 40, 80, and 120 leafhoppers per $0.21m^2$, and resistant alfalfa had levels twice as high: 0, 80, 160, and 240 leafhoppers per $0.21m^2$. Although the leafhopper number was twice as high in resistant cages, there were no differences among loss rates for all alfalfas. Again, this lack of difference suggests that resistant alfalfas outperformed the susceptible control by tolerating twice as many potato leafhoppers.

Another trend that emerged from this series of experiments was for loss rates to decrease (become more resistant) with alfalfa age. This was especially obvious in the control (Table 2). The loss rates for the control were 0.334 ± 0.224 and 0.613 ± 0.032 for the first and second cuttings of the seeding year. These values decreased to 0.095 ± 0.099 during the second year, and decreased again to 0.024 ± 0.068 during the third year. Pair-wise t-tests of all combinations showed the seeding-year, second-cutting loss rate (0.613 ± 0.032) was significantly higher than loss rates from all other age classes. A conservative interpretation of these results is that the potential for alfalfa loss from potato leafhopper is likely different between the seeding year and years after.

A similar trend for decreasing loss rates with plant age was detected in resistant alfalfa. Similar pair-wise t-tests were performed on resistant alfalfa across age classes. Data for resistant alfalfa were pooled by trial and linear regression models were fit. The experimental alfalfa was excluded from this analysis because it will not be commercialized. There were no statistical differences in the loss rates of resistant alfalfa among years.

However, the ability to tolerate the potato leafhopper increased greatly after the first cutting of the seeding year, and a trend for greater tolerance of the pest occurred in years two and three (Table 2).

Discussion

These results do not support the antibiotic resistance mechanism reported by Brewer et al. (1986a), Elden and Elgin (1992), and Elden and McCaslin (1997) in perennial glandular-haired alfalfa. The present study builds on earlier findings by combining aspects of free-choice and no-choice experimental designs and approximating field conditions. The cages forced leafhoppers to survive on a fraction (approximately 80 plants per cage at seeding) of the alfalfa plant population. Although earlier studies demonstrated that antibiosis functioned at an individual plant level, these results show it does not function when leafhoppers are caged on a heterogeneous population of resistant alfalfa. Alone, these findings cannot be used to determine the mechanism of resistance nor predict the effect of resistance on the leafhopper population. The cages prevented insects from emigrating, and the free-choice studies, mentioned earlier, showed leafhopper nonpreference.

Painter (1951) described how the role of the plant was more important than the role of the insect when tolerance was the resistance mechanism. In this study, tolerance is the best explanation of the resistance mechanism. However, it seems the important factor is the insect's response to the genetic variability in an alfalfa stand. Combining results from the present study with the feeding and sampling studies described earlier, it seems that stand-tolerance is the best explanation of the resistance mechanism.

A mechanism that appeared antibiotic using no-choice tests and single stems may appear as nonpreference when susceptible hosts are available. Brewer et al. 1986a and Elden

and Elgin 1992 confirmed this. An alfalfa stand presents leafhoppers with a diverse array of feeding and oviposition locations because of its autotetraploid genetic condition (McCoy and Bingham 1988). Therefore, while antibiosis and nonpreference may function among individual plants in a field, the fraction of suitable hosts in a resistant stand may be great enough that the stand's carrying capacity remains unchanged. It seems Hogg et al (1998) and Lefko et al. (in press) confirmed this in field studies.

Tolerance may best describe how these new alfalfas respond to feeding from the potato leafhopper. Moreover, it may function at the individual plant level, as Painter (1951) described it, or at a stand level. Below are explanations of how tolerance may function at each level.

First, plant-level tolerance, or resistance to hopperburn (Jarvis and Kehr 1966; Kindler and Kehr 1973), may exist in the alfalfa population, and its efficacy is likely variable among individual plants in a stand. One explanation is related to insect behavior. Hunter and Backus (1989) identified different feeding behaviors of the potato leafhopper and linked the symptoms with one feeding behavior (multiple-cell laceration and flush). It may be that the morphology of glandular-haired alfalfa causes the insect to change its feeding behavior to one less damaging (Brewer et al. 1986b, Calderon and Backus 1992). Another explanation is that tolerant alfalfa may metabolize or be less receptive to the toxic compounds in leafhopper saliva that causes cell damage.

Stand-tolerance can also be explained in at least two ways. Both explanations assume leafhopper damage is concentrated on suitable (less-resistant) plants in the alfalfa stand. First, the growth rate or form of resistant plants may compensate for neighboring plants that are more attractive hosts, and consequently stunted by the leafhopper (Hutchins et al. 1989b,

1990). Another explanation is that loss per potato leafhopper decreases as the number per plant increases. Leafhoppers remove assimilates, and enzymes deposited in vascular tissue during feeding causes damage (Ecale and Backus 1995a, 1995b). Hower and Flinn (1986) described how the probability of feeding on previously damaged tissue increases with insect number and showed that loss per leafhopper nymph decreases as the nymph number per plant increases. Womack (1984) corroborated these findings in a physiological study; alfalfa photosynthesis and transpiration declined as the leafhopper number per stem increased. Therefore, the leafhopper population-damage-potential might be reduced if leafhoppers aggregate on a fraction of the stand.

Studies investigating the distribution of leafhopper symptoms in an alfalfa population could provide a more definitive explanation of stand-tolerance. Additionally, the level of resistance (including nonpreference, antibiosis, and tolerance) will undoubtedly increase in future alfalfas, and the fraction of suitable hosts in the stand will likely decline. Therefore, stand-tolerance may be an artifact of the early stage of breeding for leafhopper resistance. It may be wise to predict the effect this change will have on the resistance mechanism and investigate the value of eliminating the fraction of the stand that will support a potato leafhopper population.

We propose the concept of stand tolerance. Stand tolerance implies the interplay of more than one resistance mechanism but emphasizes the impact this tactic will have on pest management by raising the economic injury level.

Calculating Economic Injury Levels

Painter (1951) pointed out how ecologically compatible and practical host plant resistance is in pest control, and he identified tolerance as a premier mechanism. Later, Stern

et al. (1959) pointed out how tolerance was unique from virtually every other pest management tactic, including other resistance mechanisms, because its objective was not to suppress the pest number. They theorized how tolerance would increase the economic injury level instead of suppressing the pest number below a tolerable level (Fig. 2). Results from this study show that stand-tolerance creates a yield advantage great enough to warrant calculating separate economic injury levels for susceptible and tolerant alfalfa. Moreover, it seems the ability to tolerate potato leafhopper changes with alfalfa age, and the rate of change may be different between resistant and susceptible alfalfa. Proposed is a two-step decision process for determining the optimal economic threshold, depending on the type and age of the alfalfa stand (Fig. 3).

The potential for loss was large and similar for both cuttings of the seeding year in the control and the first cutting in resistant alfalfa (Table 2). Therefore, the same economic threshold could be used for susceptible and resistant alfalfa during these times. These yield-loss data for these time periods were pooled and a linear model was fit to estimate the coefficients for the EIL equation ($Y = 0.456X - 2.702$). The resulting economic threshold is 8 adult leafhoppers per 10 sweeps (Fig. 3).

The loss rate for the control decreased greatly from the first and second cutting of the seeding year to the second cutting of the third year (Table 2). This difference may warrant the use of a higher economic threshold. An economic threshold of 33 leafhoppers per 10 sweeps was calculated using the yield loss coefficients for the second cutting of the second year ($Y = 0.095X$). This value is large compared to values described by Cuperus et al. (1983) (5 adults per 10 sweeps) and DeGooyer et al. (1998) (11 adults per 10 sweeps). One explanation for this is that these previous studies did not account for stand age. A

conservative economic threshold may be between 8 and 33 adult leafhoppers per 10 sweeps for alfalfa after the seeding year.

A separate economic threshold for tolerant alfalfa was calculated using pooled results from 5347LH, AmeriGuard 301, and Trailblazer, during the second cutting of the second year ($Y = 0.046X - 0.806$). This value was 80 leafhoppers per 10 sweeps, and was ten times larger than the earlier cutting of the same year and 2.4 times larger than the control in year two (Fig. 3). Inclusion of third-year data would have increased the threshold to over 1800 leafhoppers per 10 sweeps. This number is probably unrealistically large because the relationship between loss and leafhopper number is curvilinear, not linear, at densities that high.

Leafhopper-tolerant alfalfa may impact alfalfa production in many ways. Results from this study showed it has a greater yield potential than susceptible alfalfa under leafhopper stress. This could increase alfalfa yield over the life of a stand, without insecticides. Moreover, a higher economic threshold should reduce the frequency of application and quantity of insecticide for the potato leafhopper. This is shown in Fig. 4, which uses data from Lefko et al. (in press) and one additional year of unpublished data. Clearly shown is the benefit of stand-tolerance through a higher economic threshold.

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Table 1. Loss rates (slope), x intercepts, and nymph population-growth results for susceptible and tolerant alfalfa.

Alfalfa	Slope	X Intercept	r^2	Nymph index
	Seeding year, first cutting[a]			
645 (control)	0.334 ± 0.224	12.6	0.53	0.22 ± 0.60b
53V63	-1.172 ± 0.483	0.0	0.66	2.76 ± 0.67a
54H69	0.407 ± 0.141	0.0	0.73	0.59 ± 0.52b
3A09	0.653 ± 0.759	19.8	0.27	1.08 ± 0.56b
	Seeding year, second cutting[a]			
645 (control)	0.613 ± 0.032a	0.0	0.99	1.85 ± 0.53
5347LH	0.061 ± 0.093b	10.1	0.18	1.58 ± 0.40
AmeriGuard 301	0.042 ± 0.099b	0.0	0.07	2.06 ± 0.35
Trailblazer	0.158 ± 0.311ab	38.8	0.12	1.61 ± 0.24
Experimental	0.075 ± 0.309ab	5.8	0.03	1.29 ± 0.27
	Second year, second cutting[b]			
645 (control)	0.095 ± 0.099	0.0	0.24	3.71 ± 0.51
5347LH	0.004 ± 0.033	12.8	0.01	3.41 ± 0.49
AmeriGuard 301	0.007 ± 0.050	0.0	0.01	3.61 ± 0.27
Trailblazer	0.021 ± 0.043	0.0	0.07	2.69 ± 0.32
Experimental	0.003 ± 0.036	0.0	0.00	2.80 ± 0.36
	Third year, second cutting[c]			
645 (control)	0.024 ± 0.068	0.0	0.04	0.28 ± 0.11
5347LH	0.053 ± 0.014	0.0	0.83	0.14 ± 0.04
AmeriGuard 301	0.050 ± 0.050	58.2	0.34	0.25 ± 0.09
Trailblazer	-0.032 ± 0.253	na	0.01	0.44 ± 0.12

Slopes and X intercepts, by year, sharing the same letter are not statistically different using $a = 0.05$.

na The X intercept was omitted from the table because the linear regression resulted in a negative slope.

[a] Levels of infestion were 0, 15, 30, and 45 leafhoppers for all alfalfa.

[b] Levels of infestion were 0, 15, 30, and 45 leafhoppers for 645 and 0, 30, 60, and 90 for all others.

[c] Levels of infestion were 0, 40, 80, and 160 leafhoppers for 645 and 0, 80, 160, and 240 leafhoppers for all others.

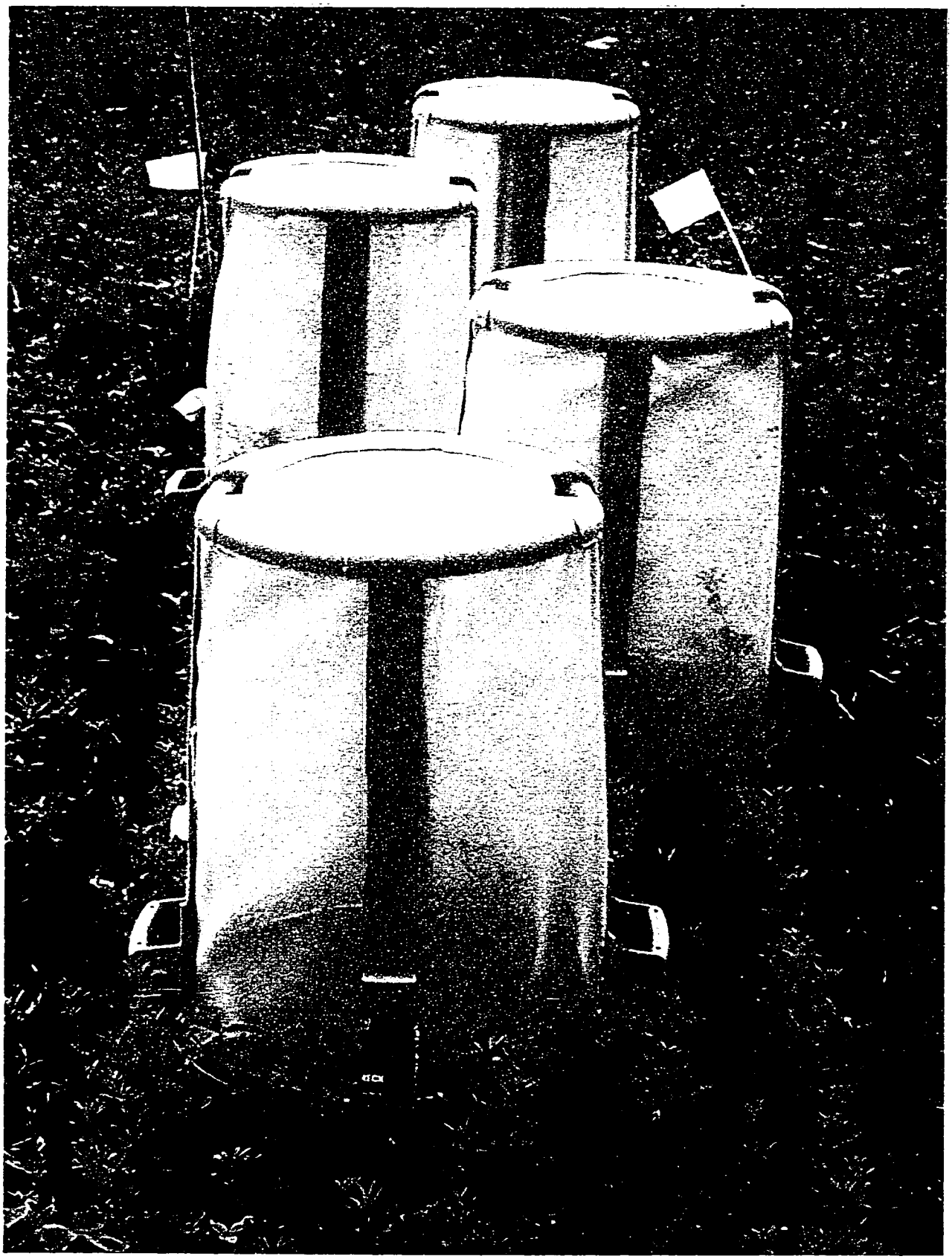
Table 2. Loss rates (slopes) for resistant and susceptible alfalfa over alfalfa age classes.

	Alflafa Type	
Alfalfa age class	Susceptible	Resistant[a]
seeding year, 1st cutting	0.334 ± 0.224a	0.455 ± 0.355
seeding year, 2nd cutting	0.613 ± 0.032b	0.053 ± 0.098
second year, 2nd cutting	0.095 ± 0.099a	0.003 ± 0.030
third year, 2nd cutting	0.024 ± 0.068a	0.020 ± 0.078

Loss rates, by alfalfa type, sharing the same letter are not statistically different using $a = 0.05$.

[a] 53V63 was dropped out of this analysis because of its original negative slope.

Figure 1. An example of cage arrangement in one alfalfa plot.



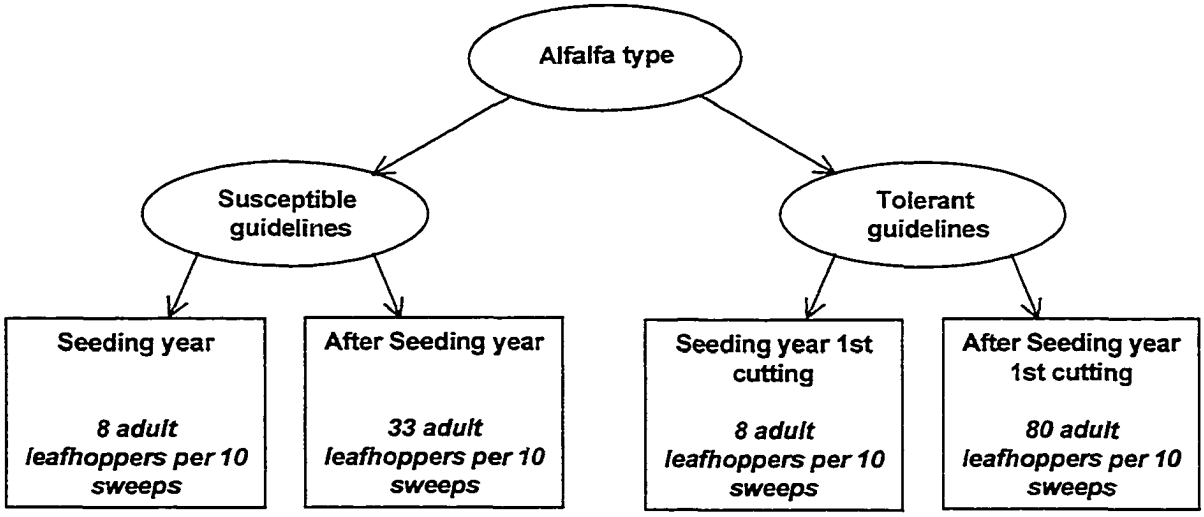


Figure 2. Two-tiered decision process for determining the economic threshold for potato leafhopper in alfalfa.

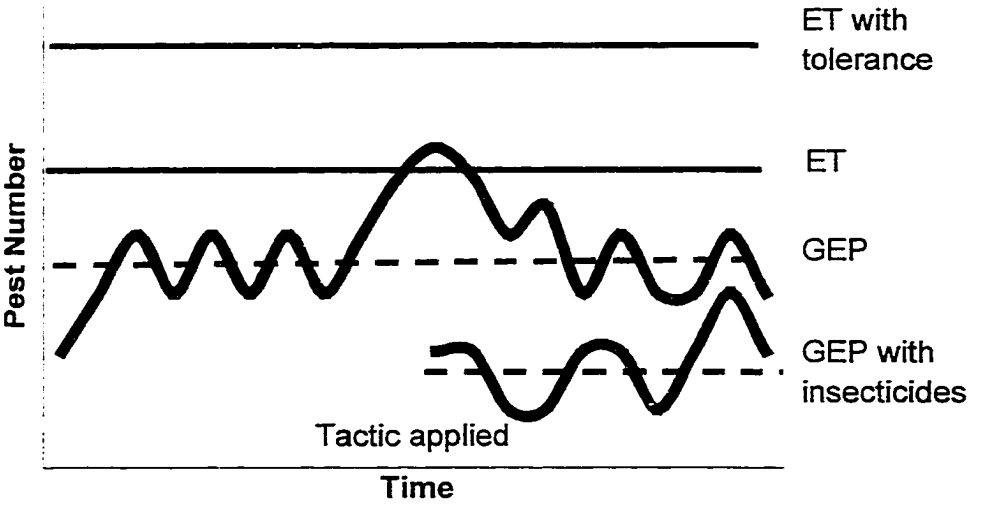


Figure 3. Stylized graph showing the effect of tolerance, compared with insecticides, on the economic threshold (ET). GEP, general equilibrium position. Modeled after figures in Stern et al. (1959).

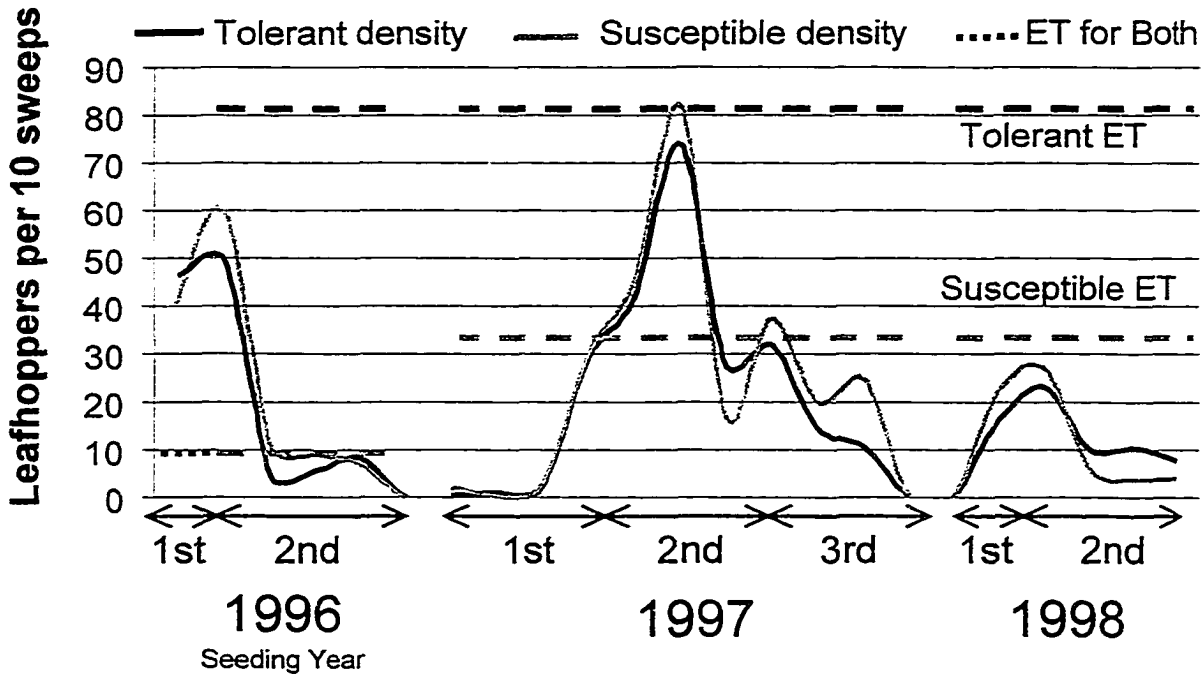


Figure 4. The benefit from tolerance through separate economic thresholds (ET) for susceptible and tolerant alfalfa. Tolerant and susceptible alfalfa have the same ET during the first cutting of 1996, and the ETs diverge beginning with the second cutting of the same year. The ET is exceeded in tolerant alfalfa only during the first cutting of 1996. The ET is exceeded in susceptible alfalfa during both cutting of 1996 and the second cutting and early part of the third cutting in 1997. The susceptible alfalfa would have spent more time under economic loss conditions if the threshold remained at a conservative 8 leafhoppers per 10 sweeps.

CHAPTER 6. SYMPTOM AND GROWTH RESPONSE OF POTATO LEAFHOPPER-TOLERANT ALFALFA TO FEEDING BY POTATO LEAFHOPPER

A paper submitted to the Journal of Economic Entomology

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Abstract

Two study areas, one near Ames, IA and one near Chariton, IA, were planted with 4 potato leafhopper-tolerant (glandular-haired) varieties and 1 susceptible variety during the spring of 1996. Estimates of alfalfa dry matter, stem length, number of nodes, and hopperburn were taken on each harvest date at each location, and the density of adult potato leafhoppers (*Empoasca fabae* Harris) in each variety was sampled regularly over the 3-year study. Comparisons of the average quantity of dry matter and the average density of potato leafhopper were made among varieties for each growth interval at each location. Estimates of stem length, node number, internodal distance, and hopperburn were compared among varieties under high and low pest pressure regimes; three growth intervals were used for each regime. There were only 2 growth intervals out 15 when the density of potato leafhopper was significantly greater in the susceptible control compared with one or more tolerant varieties. Although there was only 1 instance when significant differences in dry matter was found among alfalfa types, there was a trend for tolerant alfalfa to produce more dry matter than susceptible alfalfa as the density of potato leafhopper increased. This trend was explained by tolerant varieties having more nodes, longer internodes, longer stems, and less hopperburn compared with the susceptible variety when the leafhopper number was high.

Introduction

Alfalfa (*Medicago* spp.) serves as host to an abundance of phytophagous insect species. One of these, the potato leafhopper, *Empoasca fabae* (Harris), is considered the primary pest of alfalfa in the north central and northeastern United States. This ubiquitous pest is an assimilate remover, and its symptoms in alfalfa are stem stunting and leaf chlorosis, commonly called hopperburn.

Economic loss from potato leafhopper is best linked to reductions in alfalfa biomass but less so of forage quality (Hutchins et al. 1989a). Reductions in biomass result from shorter stems and not lower stem density or lower leaf mass (Hutchins and Pedigo 1989b; Oloumi-Sadeghi et al. 1988; Hower and Flinn 1986; Faris et al. 1981). Hutchins and Pedigo (1989b) found that high leafhopper pressure reduced the number of nodes per stem, and this reduction may have contributed to lower leaf area indices in leafhopper-stressed alfalfa. They also suggested that reduced stem length may provide less opportunity for alfalfa leaves to compete with weeds for photosynthetically active radiation, and that this may favor the growth of weed species and reduce the life of an alfalfa stand.

In 1997, several seed companies sold alfalfa varieties that were advertised as resistant to the potato leafhopper. How these varieties outperform susceptible alfalfa when the leafhopper number is high is still being investigated. Some of the most significant results have shown potato leafhopper densities are usually similar in resistant and susceptible alfalfa types (Hogg et al. 1998; Lefko et al. in press a). Lefko et al. (in press b) described this result using a stand-tolerance concept; the density of potato leafhopper is similar between tolerant and susceptible alfalfa because leafhoppers are surviving on a fraction of the alfalfa stand that is most favorable to them. In the same study, they showed that tolerant alfalfa can suffer

loss from potato leafhopper; however, the potential for loss is significantly less than in susceptible alfalfa. Besides this study, there are few reports of yield benefits from leafhopper-tolerant alfalfa. Hogg et al. (1998) showed that tolerant varieties had slightly lower yields compared with the susceptible variety when leafhopper pressure is low and a yield advantage when pest pressure is high and populations were left unmanaged.

To date, there are no reports of how the growth and developmental characteristics of leafhopper-tolerant alfalfa differ from susceptible alfalfa when under stress from potato leafhopper. An explanation of these characteristics may help researchers understand how tolerant alfalfa outperforms susceptible alfalfa under high potato leafhopper pressure. The objectives of this study were to (1) compare the symptoms of potato leafhopper in tolerant and susceptible alfalfa types, and (2) determine the alfalfa developmental characteristics responsible for the yield advantage from tolerant alfalfa.

Materials and Methods

Two study areas, one near Ames, IA, and one near Chariton, IA, were planted with 4 potato leafhopper-tolerant (glandular-haired) varieties and 1 susceptible variety. These locations were chosen based on environmental differences and histories with different pest populations. Tolerant varieties included ABI AmeriGuard 301®, CENEX Trailblazer®, Pioneer Hi-Bred 5347LH®, and XAE49, a Pioneer Hi-Bred experimental line. The susceptible control was Garst 645®, a variety that, historically, has produced high yield test results in Iowa. The 5 varieties were arranged according to a randomized complete block design with 4 replications. Both locations were planted during the fourth week of April 1996 and treated with Eptam® 1 week before planting to reduce competition from weeds. Each plot measured 7.6 m long by 6.1 m wide and was planted by using a single-row planter; these

dimensions were determined by the availability of seed. Rows were spaced 20 cm apart, and the planter was calibrated to 0.19 g of seed per meter (10 lb. per acre). The plots at both sites were located within 0.6 ha fields of Defiant®, a susceptible variety of alfalfa.

Alfalfa was harvested twice during 1996 and 3 times during 1997 at each location. Alfalfa was harvested 3 times during 1998 at the Chariton location; however, inclement weather delayed the second harvest and prevented a third harvest at the Ames location. Alfalfa was harvested when it reached approximately the 0.1-bloom stage of development. Dates of alfalfa harvest are given in Table 1.

Estimates of alfalfa dry matter, stem length, number of nodes, and hopperburn were taken on each harvest date. Dry matter sampling programs were different between 1996 and subsequent years. Rows of alfalfa were distinct during 1996; therefore, dry matter was estimated by sampling a randomly selected 0.5 m of row in each plot. In 1997 and 1998 rows were less distinct, and dry matter was estimated from 4 0.35 m^2 quadrats randomly placed in each plot. After stem length, node number, and hopperburn measurements were taken, dry matter samples were bagged and dried at 60° C for 72 hr. Dry matter of alfalfa from each cage was weighed immediately after the drying period.

In 1996, stem length, node number, and hopperburn measurements were taken from all stems collected for dry matter estimates. In 1997 and 1998, the same data were collected from 5 randomly selected stems from each of quadrat per plot (20 stems per plot, 80 stems per alfalfa). Stem length was measured from the clipped end to terminal end, which was usually a bud. Nodes were counted from the clipped end to the last node with a fully expanded trifoliolate. Internodal distance was calculated for each stem by dividing length by the node number. A 1–5 hopperburn scale was used to estimate the severity of this symptom.

The scale was: 1) no hopperburn, 2) no more than 1/3 of leaves with yellowing, 3) between 1/3 and 2/3 of leaves with yellowing, 4) more than 2/3 of leaves with yellowing, and 5) more than 2/3 of leaves with yellowing and some with necrotic tissue.

Stem length, node number, internodal distance, and hopperburn were compared among varieties under two different pest pressure regimes. Potato leafhopper density data from 8 July 1996 Ames, 10 July 1996 Chariton, and 1 July 1997 Ames, represented the high pest pressure regime (Table 1) (AmeriGuard 301 n = 358, Trailblazer n = 361, 5347LH n = 364, XAE49 n = 342, 645 n = 392). The average pest density ranged from 2.2 to 5.0 potato leafhoppers per sweep for every variety during these growth intervals. Stem data from these 3 growth intervals were pooled for each variety and used for the high pest pressure regime. The potato leafhopper density was very low during the 22 May 1997 Ames, 29 May 1997 Chariton, and 19 May 1998 Chariton, growth intervals (Table 1). These stem data were pooled and used as the low pest pressure regime (n = 240 for each variety). During both regimes, there was no growth interval when the number of potato leafhoppers differed significantly among varieties. Separate analyses of variance tests were used to determine differences in stem characteristics among varieties under high and low pest pressure regimes. If the analysis of variance was significant ($P = 0.05$), means were separated using least significant difference analysis ($P = 0.05$). Alfalfa yield typically declines with each harvest (7:5:3) (Fick et al. 1988). The differences in stem growth associated with this decline precluded comparisons of stem variables between pest pressure regimes for individual varieties (Nelson and Smith 1968).

Insect sampling methods were similar in 1996, 1997, and 1998. Potato leafhopper adults were sampled using a 38 cm-dia. muslin sweepnet. A sampling unit consisted of 12

pendulum sweeps of the alfalfa canopy in a plot. Sweep samples were taken lengthwise through plots with sampling lanes within plots alternating with sampling date. Insect samples were bagged and kept frozen until processed. The number of sampling dates during each regrowth interval at each location is given in Table 1.

The density of potato leafhopper adults and nymphs was compared among varieties according to growth intervals. Data from all insect sampling dates during the growth interval were pooled in the analysis of variance tests. Means were separated using least significant difference analysis ($P = 0.05$) if significant differences in leafhopper density were detected among varieties.

Results and Discussion

Overall, the density of adult potato leafhopper was similar among all varieties throughout the 3-year study at both locations (Table 1). There were two instances when the leafhopper was more dense in the susceptible control compared with leafhopper tolerant varieties. Leafhoppers were significantly more dense in the susceptible variety (645) compared with all tolerant varieties during the second cutting of 1998 at the Ames location. Also, leafhoppers were significantly more dense in 645 compared with XAE49 during the second growth interval of 1997.

There was only one instance when the quantity of dry matter differed significantly among alfalfa types. There was significantly more dry matter produced in 645 compared to AmeriGuard 301, Trailblazer, and 5347LH during the Ames 2 July 1997 growth interval. The leafhopper density was moderate in all varieties during that interval; however, it was numerically higher in 645. Dry matter results should not be used to extrapolate estimates of alfalfa yield per production area because the sampling units were small.

Seemingly, there was a tendency for the dry matter estimate in the susceptible alfalfa to exceed the dry matter estimate in tolerant varieties when the leafhopper number was low. Conversely, the quantity of dry matter in tolerant alfalfa often exceeded the quantity of dry matter in susceptible alfalfa when the leafhopper number was high. Linear regression was used to compare dry matter production in the tolerant varieties, compared with the susceptible variety, under different pest pressures. The quantity of dry matter for each tolerant variety was converted to a percentage of the dry matter produced by the susceptible control during the same growth interval. This value was calculated for all 60 varieties by growth interval by location combinations. Positive values indicated a tolerant variety produced more dry matter than the susceptible variety, and values less than zero indicated greater dry matter in the susceptible variety. These percentages were plotted against the average adult leafhopper density (Table 1) for the respective variety and growth interval, and a linear model was fitted (Fig. 1) (SAS 1990). The linear model was $Y = 0.50x - 10.04$, $r^2 = 0.09$, and the slope was significantly different from 0 ($F = 6.00$, $df = 1,59$, $P = 0.02$). Although this model has poor predictive value, the relationship was strongly positive. Therefore, the potential for tolerant alfalfa to produce more dry matter per unit area than susceptible alfalfa increases as the leafhopper density increases. The true x intercept is likely between 0 and 20 leafhoppers per 12 sweeps, and most percentages were negative below a leafhopper density of 20 leafhoppers per 12 sweeps. It seemed that tolerant varieties produced slightly less dry matter than the susceptible variety (645) when the leafhopper density was low. These findings are consistent with results reported by Hogg et al. (1998). The differences in the number of nodes, stem length, internodal distance, and hopperburn, among alfalfa types can explain an important part of this relationship.

There were differences in the average number of nodes among varieties under both high and low pest pressure regimes. Under the low pressure regime, there were significantly fewer nodes in Trailblazer, 5347LH, and the susceptible 645 compared with the others (Fig. 2). Although significant, these differences are all less than 1 node and relatively less important. Under the high pest pressure regime, there were significantly fewer nodes in the susceptible variety compared with all tolerant varieties. Tolerant varieties had as many as 1.3 more nodes per stem compared with the susceptible. The ability of potato leafhopper-induced injury to reduce the number of nodes was demonstrated earlier by Hutchins and Pedigo (1989b). They found as many as 2.2 more nodes on alfalfa not stressed by the leafhopper compared with alfalfa that was stressed by 200 leafhoppers per 1 m^2 1 day after harvest. Each additional node would increase the stem length by the length of 1 internode. Moreover, it would increase the trifoliolate per plant number over the course of development. These changes would increase the cumulative leaf area and perhaps increase the crop growth rate, consequently, increasing the yield potential.

Internodal distance is a function of stem length and number of nodes, and there were differences among varieties under both pressure regimes. Under the low pressure regime, 645 and 5347LH had internodal distances significantly greater than three tolerant varieties (Fig. 3). This may be a partial explanation for why tolerant varieties, in general, produced less dry matter compared with the susceptible variety when the leafhopper number was low. Under the high pressure regime, 5347LH and XAE49 had internodal distances greater than all other varieties. These differences are small but meaningful because the number of nodes per stem ranged from 8 to 10. And as already described, all tolerant varieties produced significantly more nodes under the heavy pest pressure regime. The ability of tolerant alfalfa

to maintain longer internodal distances (resist stunting), compared with the susceptible alfalfa, contributed to its yield advantage when the leafhopper number was high.

The additional nodes and longer internodal distances were likely the primary causes for large differences in stem length among tolerant and susceptible varieties. There were significant differences in the length of stems among varieties under both pest pressure regimes. Under low pressure, 645 had an average stem length significantly greater than three tolerant varieties (Fig. 4). Again, this helps explain why dry matter was often higher in the susceptible variety when the leafhopper number was low (Fig. 1). Conversely, under the high pressure regime there were three tolerant varieties that had average stem lengths greater than the susceptible variety. Several studies have shown how leafhopper density has a direct effect on alfalfa stem length and that stem length is the plant component that is most responsible for variation in yield (Hutchins et al. 1989a; Hutchins and Pedigo 1989b; Oloumi-Sadeghi et al. 1988). Considering the density of potato leafhopper was similar among varieties, it seems that tolerant alfalfa has the potential to grow longer stems than susceptible alfalfa when both are under high leafhopper pressure. This characteristic is probably most responsible for the increased yield potential, described by Hogg et al. (1998) and Lefko et al. (in press b), of tolerant varieties when under leafhopper stress.

Comparisons of hopperburn under the high pest pressure regime showed dramatic differences between resistant and susceptible alfalfa types. Under high pressure, the hopperburn score in the susceptible variety was significantly greater compared with all tolerant varieties (Fig. 5). There were no significant differences in the average hopperburn score among tolerant varieties; however, it is important to note that tolerant alfalfa did show the hopperburn symptom.

The potato leafhopper density was similar among alfalfa types during these regrowth intervals; therefore, is seems the tolerant varieties resisted hopperburn. This trait was identified earlier by Jarvis and Kehr (1966), who screened 75 alfalfa clones for resistance to potato leafhopper. They found that equal numbers of nymphs did not cause the same degree of hopperburn. Similarly, Kindler and Kehr (1973) screened alfalfa clones and found variable levels of alfalfa resistance to hopperburn and poor relationship between hopperburn and final alfalfa yield.

Resistance to hopperburn could increase the yield and the feed value of tolerant alfalfa compared with susceptible alfalfa when under leafhopper stress. Fuess and Tesar (1968) found that leaf-drop, due to natural leaf senescence, was increased by 1.2 Mg ha^{-1} when the harvest maturity was delayed from 10 to 100% bloom. The hopperburn symptom mimics natural leaf senescence, and hopperburned leaves tend to drop from stems before healthy leaves. Leaf-drop increases greatly after alfalfa is cut and the moisture content begins to decline. Therefore, alfalfa that resists hopperburn may maintain more leaves and increase total yield. Moreover, a higher leaf fraction would increase the feed value of that alfalfa.

Data on alfalfa phenology was not collected during this study; however, observations were made on the days to harvest maturity for both alfalfa types. Tolerant alfalfa matured 7 to 10 days earlier than susceptible alfalfa after the initial seeding and during growth intervals when the leafhopper density was particularly high (Table 1, 1 July 1997, Ames). It is conceivable that this resistance trait could shorten the alfalfa growth interval enough in areas where the leafhopper is a perennial pest that some producers limited to 3 cuttings could obtain 4 cuttings.

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Table 1. Average dry matter estimates ±SEM and corresponding average density ±SEM (# per 12 sweeps) of adult potato leafhoppers (PLH) in alfalfa treatments.

	Ames, IA					Chariton, IA				
	AmeriGuard 301, ABI	Trailblazer, CENEX	5347LH, Pioneer	XAE49, Pioneer	645, Garst	AmeriGuard 301, ABI	Trailblazer, CENEX	5347LH, Pioneer	XAE49, Pioneer	645, Garst
	8 July 1996 (n = 2)[c]					*10 July 1996 (n = 2)*[c]				
Alfalfa dry matter[a]	10.45 ± 2.53	11.95 ± 2.09	18.34 ± 3.17	14.39 ± 1.48	13.54 ± 1.53	19.44 ± 2.75	18.15 ± 2.70	21.46 ± 1.71	17.59 ± 3.42	16.62 ± 0.86
PLH density	36.1 ± 9.3	26.8 ± 9.2	57.6 ± 10.9	40.4 ± 7.7	59.9 ± 18.5	48.3 ± 20.0	46.8 ± 17.2	49.0 ± 16.9	36.9 ± 16.8	37.0 ± 13.9
	28 August 1996 (n = 3)[c]					*29 August 1996 (n = 6)*[c]				
Alfalfa dry matter[a]	12.54 ± 2.36	16.84 ± 2.58	18.52 ± 1.46	15.09 ± 2.21	12.99 ± 1.08	16.4 ± 2.22	15.77 ± 2.82	19.78 ± 2.15	18.37 ± 2.48	21.97 ± 3.05
PLH density	6.1 ± 1.3	5.7 ± 1.0	7.6 ± 0.9	6.7 ± 1.5	10.7 ± 2.6	8.3 ± 1.0	6.3 ± 0.8	13.4 ± 2.2	7.2 ± 1.2	8.3 ± 1.5
	22 May 1997 (n = 2)[c]					*29 May 1997 (n = 3)*[c]				
Alfalfa dry matter[b]	19.64 ± 3.27	19.59 ± 3.55	21.57 ± 2.07	19.72 ± 3.12	14.51 ± 2.93	18.00 ± 1.89	19.45 ± 2.32	18.54 ± 2.53	21.17 ± 1.68	25.34 ± 4.47
PLH density	0.9 ± 0.5	1.3 ± 0.7	0.1 ± 0.1	0.6 ± 0.3	1.3 ± 0.8	1.2 ± 0.5	1.7 ± 0.8	0.7 ± 0.3	2.0 ± 0.7	1.2 ± 0.4
	1 July 1997 (n = 5)[c]					*2 July 1997 (n = 4)*[c]				
Alfalfa dry matter[b]	31.70 ± 4.54	26.69 ± 2.60	36.25 ± 5.74	31.87 ± 5.43	27.9 ± 4.11	24.16 ± 1.96 b	24.79 ± 1.86 b	25.09 ± 2.14 b	27.91 ± 0.69 ab	33.09 ± 1.51 a
PLH density	42.5 ± 9.2	32.1 ± 7.1	35.6 ± 7.7	32.7 ± 6.4	38.4 ± 9.4	12.4 ± 3.0 ab	10.4 ± 1.7 ab	11.7 ± 2.4 ab	9.6 ± 2.1 b	18.1 ± 3.3 a
	18 August 1997 (n = 5)[c]					*12 August 1997 (n = 3)*[c]				
Alfalfa dry matter[b]	20.44 ± 1.43	22.53 ± 0.93	22.86 ± 3.03	20.47 ± 2.39	16.53 ± 1.17	15.93 ± 1.11	18.34 ± 1.03	17.43 ± 1.30	19.64 ± 1.98	20.48 ± 1.12
PLH density	21.1 ± 3.3	18.8 ± 4.1	20.6 ± 3.5	17.5 ± 3.2	24.0 ± 3.6	6.7 ± 1.0	4.3 ± 0.9	7.9 ± 1.6	5.3 ± 0.8	8.8 ± 2.2
	26 May 1998 (n = 3)[c]					*19 May 1998 (n = 1)*[c]				
Alfalfa dry matter[b]	28.15 ± 3.10	31.52 ± 3.81	34.84 ± 1.11	32.83 ± 4.15	32.35 ± 5.38	24.46 ± 3.94	23.94 ± 4.01	26.69 ± 1.32	26.86 ± 4.27	28.47 ± 1.65
PLH density	21.0 ± 4.85	17.1 ± 4.15	19.5 ± 4.61	21.4 ± 5.47	15.8 ± 3.73	1.3 ± 0.8	3.3 ± 0.8	4.3 ± 2.3	5.3 ± 1.7	5.0 ± 1.7
	29 July 1998 (n = 3)[c]					*8 July 1998 (n = 5)*[c]				
Alfalfa dry matter[b]	19.26 ± 4.72	21.11 ± 6.50	25.19 ± 1.16	21.67 ± 3.77	26.26 ± 3.81	21.44 ± 2.04	24.1 ± 9.10	21.75 ± 4.32	21.32 ± 5.91	34.7 ± 6.00
PLH density	6.5 ± 1.33 b	4.2 ± 1.07 b	6.3 ± 1.43 b	4.9 ± 0.79 b	11.3 ± 1.70 a	13.6 ± 3.2	12.0 ± 3.6	15.2 ± 2.8	8.7 ± 1.4	17.3 ± 2.7
						19 August 1998 (n = 4)[c]				
						9.3 ± 1.66	10.02 ± 1.73	9.25 ± 2.53	9.87 ± 1.44	11.23 ± 0.67
						8.6 ± 1.7 a	4.5 ± 0.7 b	8.4 ± 1.7 ab	4.5 ± 0.9 b	8.1 ± 1.8 ab

[a] Dry matter estimates were based on 0.5 m of row, n = 4 for each treatment and sampling date.

[b] Dry matter estimates were based on 0.35 m^2 quadrats.

[c] n equals the number of PLH sampling dates during the alfalfa growth interval.

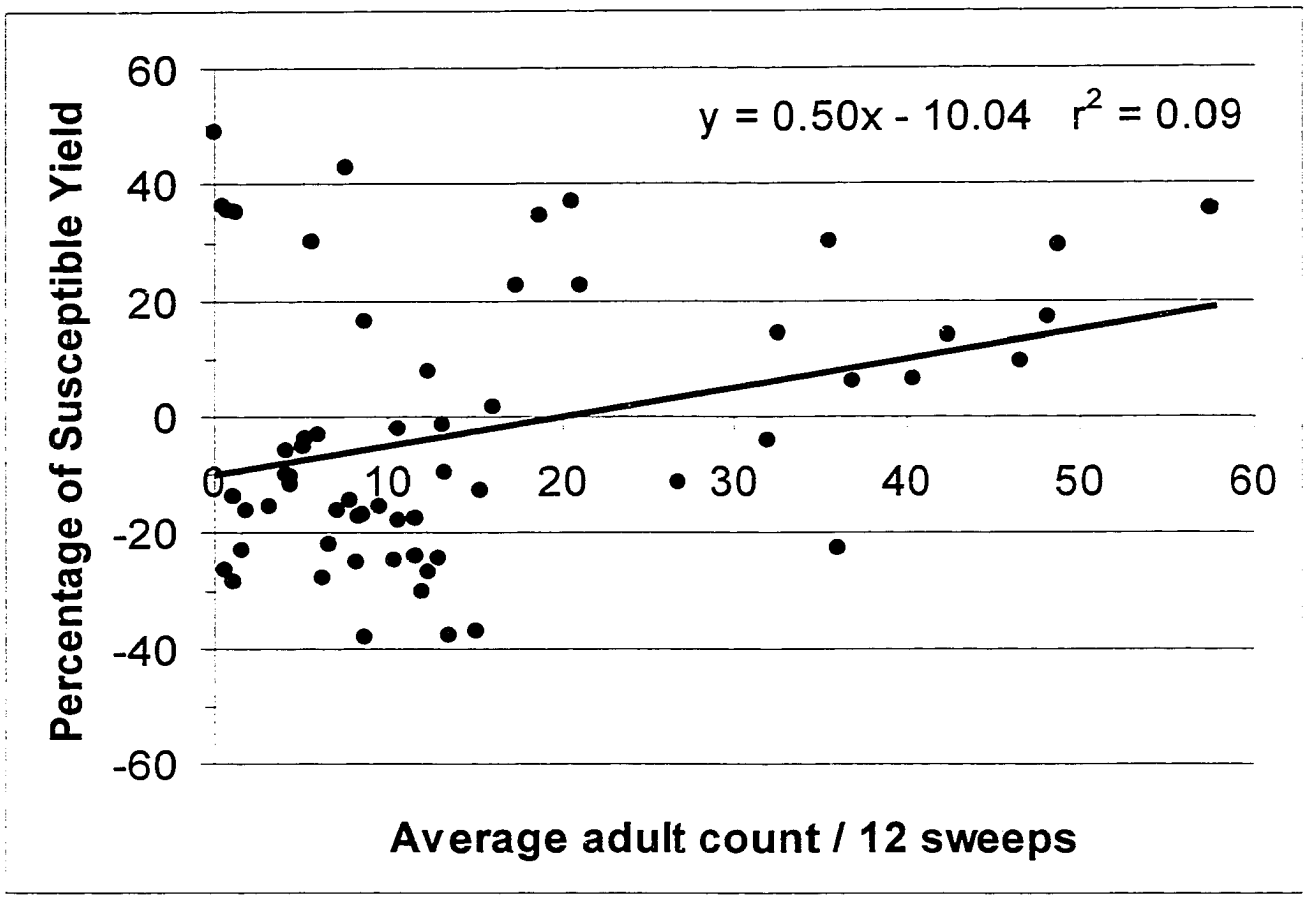

Figure 1. Dry matter yield of tolerant varieties expressed as percentages of the dry matter yield of the susceptible alfalfa in the same experiment (n = 60).

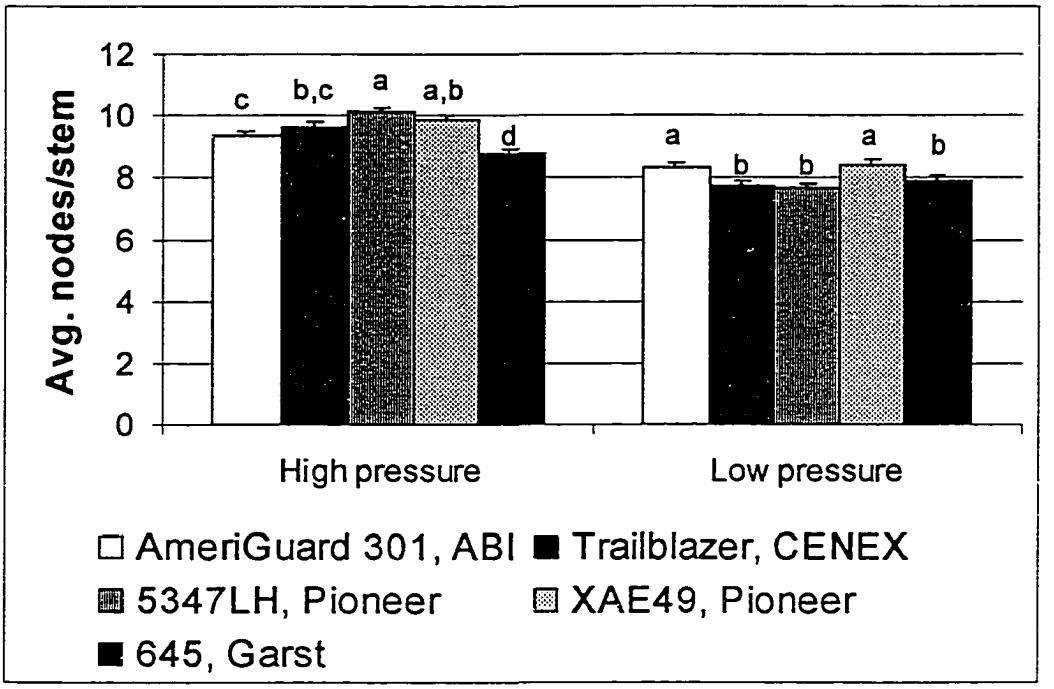

Figure 2. Average number of nodes ±SEM in all varieties under low and high pest pressure regimes.

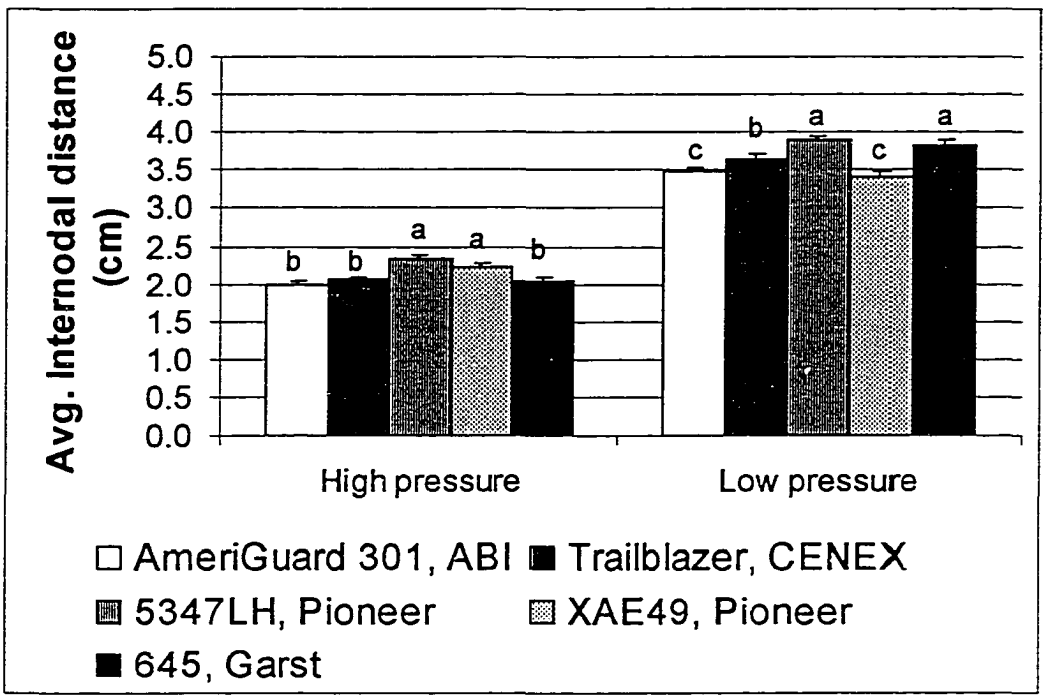

Figure 3. Average length of internodes ±SEM in all varieties under low and high pest pressure regimes.

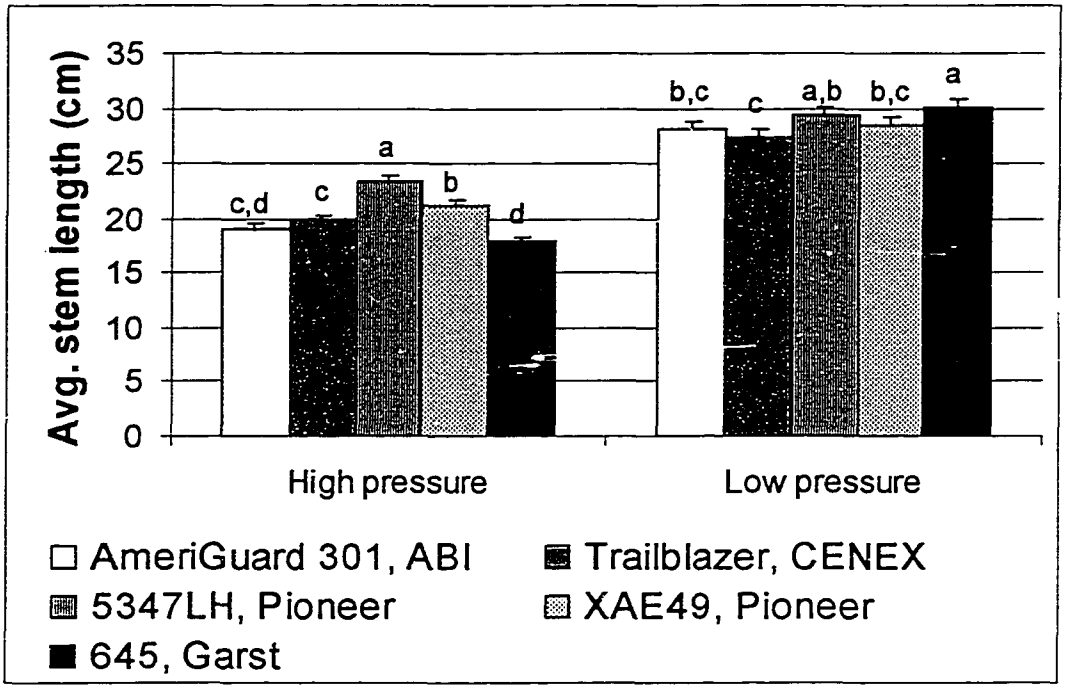

Figure 4. Average stem length ±SEM for all varieties under high and low pest pressure regimes.

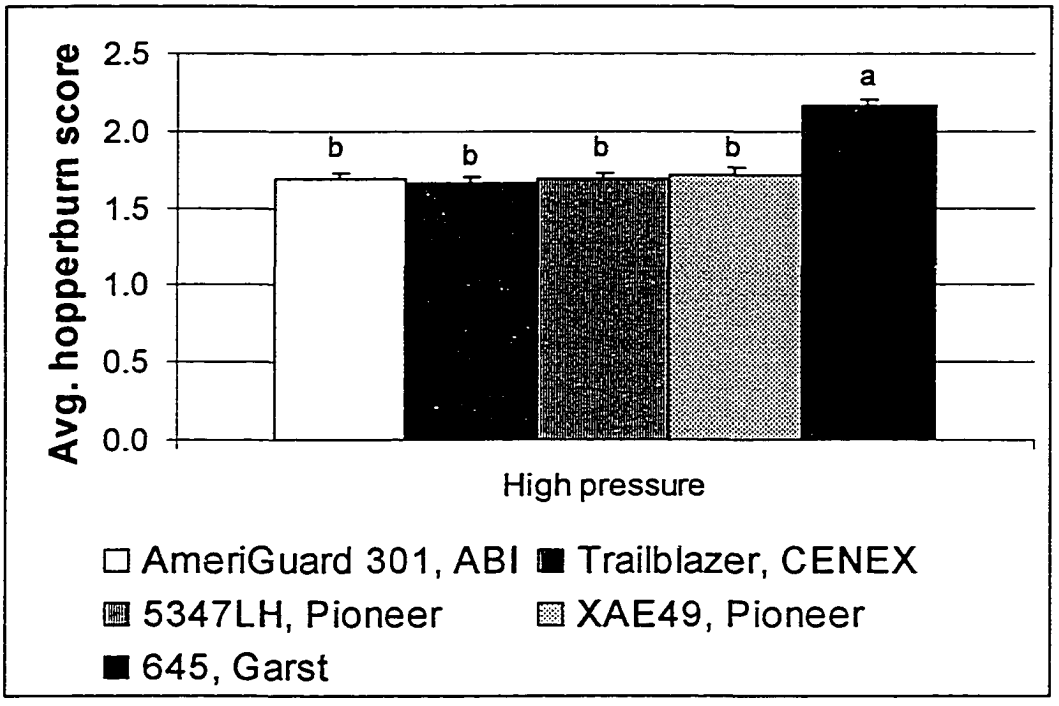

Figure 5. Average hopperburn score ±SEM for all varieties under the high pest pressure regimes.

CHAPTER 7. GENERAL CONCLUSIONS

In 1997 several seed companies released alfalfa products that were marketed as resistant to the potato leafhopper, (*Empoasca fabae* Harris), the key pest of this crop in the Midwest and northeastern United States. The specific objectives of this dissertation were 1) to determine the effect potato leafhopper-resistant alfalfa has on the insect community, 2) to investigate the presence and relative importance of antixenosis, antibiosis, and tolerance in leafhopper-resistant alfalfa, and 3) to determine if the alfalfa yield response to potato leafhopper was different between resistant and susceptible alfalfa. This difference was detected, therefore, the fourth objective was to determine the growth characteristics of resistant alfalfa that allowed it to outperform susceptible alfalfa. This information is critical in order to determine how pest and potential natural enemy populations will respond to the mechanism of resistance in new resistant varieties. Potential insect-alfalfa interactions such as multiple pest resistance, pest or natural enemy displacement, or no interactions at all, may change pest management guidelines in potato leafhopper-resistant alfalfa.

The first objective, to determine the effect potato leafhopper-resistant alfalfa has on the insect community, was investigated with a field study that compared the insect communities of 4 resistant alfalfas and 1 susceptible alfalfa. Plots were planted in 1996 and the insect communities were sampled from planting until the fall of 1997. Eighteen insect species, including pests and potential natural enemies, were sampled throughout the study. Only 4 pest species were significantly more dense on a given date in the susceptible alfalfa compared with any resistant variety throughout the 2-yr study. Six pest species were significantly more dense in resistant varieties compared with the susceptible control in 17 species-by-date-by-variety comparisons, and the same species were never significantly less

dense in susceptible alfalfa. Adult potato leafhopper density was significantly greater in the susceptible control compared with all resistant varieties in only 11 of the 140 date-by-variety comparisons. The density of potato leafhopper nymphs was significantly greater in the susceptible control in only 4 of the 92 date-by-variety comparisons. Potential insect natural enemies were significantly more dense in resistant alfalfa compared with the susceptible control in 11 species-by-date-by-variety comparisons, and significantly less dense in only 1. Results from this study show that the insect communities in these first generation leafhopper-resistant alfalfa varieties likely will not differ from those found in susceptible alfalfa. Potato leafhopper, and several other pests and potential natural enemies, showed no preference for field plots of susceptible alfalfa compared with potato leafhopper-resistant varieties of alfalfa.

The second objective, to investigate the presence and relative importance of antixenosis, antibiosis, and tolerance in leafhopper-resistant alfalfa, was investigated using a series of laboratory and field studies. Although the potato leafhopper showed no preference for susceptible alfalfa in the field, they did show a preference for susceptible alfalfa in laboratory free-choice studies. When given a choice between a resistant cutting and a susceptible cutting leafhoppers tended to feed more on the susceptible alfalfa. This tendency increased as cuttings from alfalfa clones with greater resistance were substituted in the free-choice feeding arenas. The reason for this preference was unclear because in no-choice feeding tests leafhoppers fed as much on highly resistant clones as they did on the susceptible control. Dense pubescence did not prevent the leafhopper from feeding, therefore, this antixenotic mechanism seemed predominantly a function of insect behavior and less so a function of the plant. The conclusion was that a heterogeneous stand of field grown alfalfa

provided enough variation that the carrying capacity was not limiting, and leafhoppers could overcome the antixenotic mechanism that was observed in the laboratory.

Antibiosis was investigated by caging populations of the potato leafhopper on field grown resistant and susceptible alfalfa and then measuring the growth of the nymph population. This study was performed 3 times on varieties from the first generation of commercial leafhopper-resistant alfalfa and once on varieties from the second generation, which to date are not available commercially. In no instance was there a difference in the nymph population that was produced on resistant and susceptible alfalfa. These results, in combination with the results of objective 1, strongly undermined the concept that an antibiotic mechanism is responsible for the increased yield potential in these new alfalfa varieties.

Tolerance was the last mechanism of resistance that was investigated and proved to be the most important mechanism. Besides sampling the nymph population in the cage study described above, estimates of alfalfa dry matter were also collected from inside the cages. Caging different leafhopper densities on alfalfa provided variable pest pressure and alfalfa dry matter results that were used to calculate linear yield loss models. Model coefficients were compared among alfalfas and among age classes for alfalfa types. The potential for resistant alfalfa to outperform susceptible alfalfa under leafhopper stress began after the initial growth of seedlings and continued through year 3 of the study. Leafhopper resistant alfalfa supported a pest population and outperformed the susceptible control; this is the definition of tolerance. The mechanism, however, was described as stand-tolerance and seemed to increase as the alfalfa stand matured.

Stand tolerance implies the interplay of more than 1 resistance mechanism, but emphasizes the impact this tactic will have on pest management by raising the economic injury level. The onset of stand-tolerance raised the economic threshold from 8 to 80 leafhoppers per 10 sweeps in leafhopper-resistant alfalfa. Another very interesting finding was that the potential for loss from potato leafhopper decreased greatly in susceptible alfalfa from the first to the second year of the stand; this could greatly increase the economic threshold in susceptible alfalfa.

The alfalfa growth characteristics that accounted for greater yield in resistant alfalfa over susceptible alfalfa were investigated. Estimates of alfalfa dry matter, stem length, number of nodes, and hopperburn were taken on each harvest date at 2 locations over the 3 year study. Although there was only 1 instance when significant differences in dry matter was found among alfalfa types, there was a trend for tolerant alfalfa to produce more dry matter than susceptible alfalfa as the density of potato leafhopper increased. This trend was explained by tolerant varieties having more nodes, longer internodes, longer stems, and less hopperburn compared with the susceptible variety when the leafhopper number was high.

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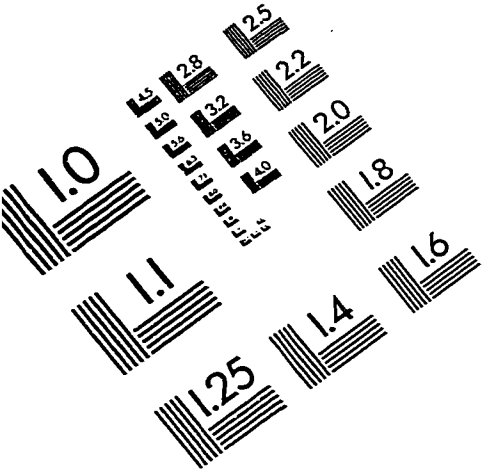

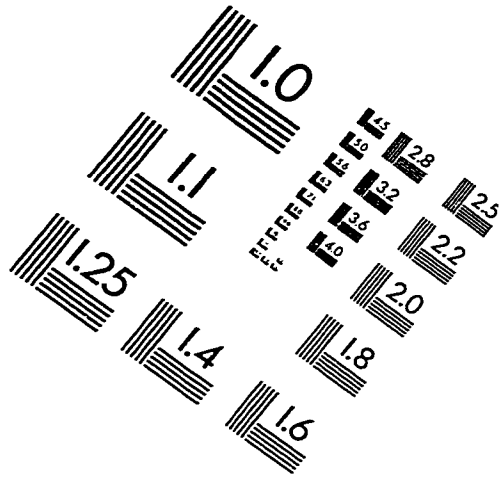

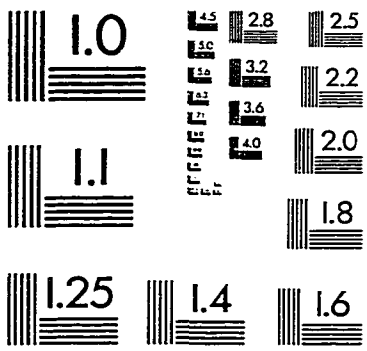

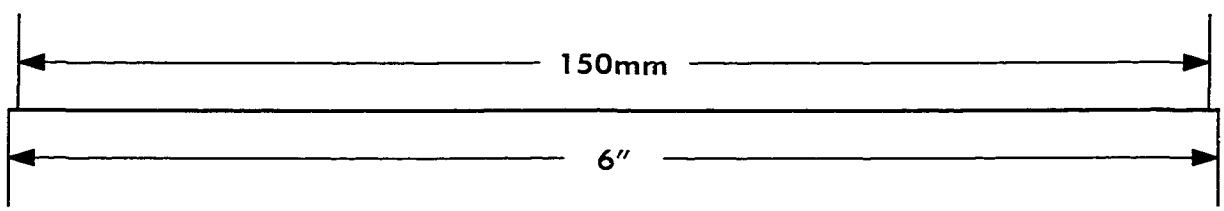

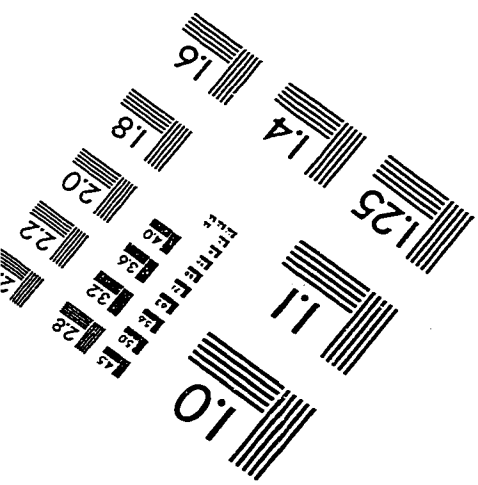

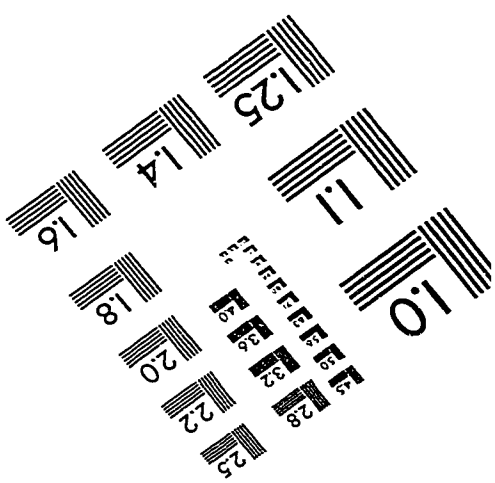

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